This Week in Virology

with **Vincent** Racaniello, Ph.D.

Episode #043: Virus Classification

Hosts: **Vincent** Racaniello and **Dick** Despommier

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Vincent: This Week in Virology, the podcast that about viruses, the kind that make you sick.

This Week in Virology, Episode 43 for August 2nd, 2009. I'm Vincent Racaniello and its Sunday and I was away again last week so I didn't have a chance to record the usual *This Week in Virology* with other hosts. However a few weeks ago Dick Despommier and myself recorded part two of *Virology 101*, *Virus Classification*. So let's listen to that now and I'll be back on the other side with some discussion of the 1976 swine flu vaccine and Guillain-Barre.

Vincent: Do you want to talk about virus classification?

Dick: Sure because I would like to know about whether or not they are classified by their shapes or their chemical characteristics or the size of their genomes or whether they are RNA or DNA viruses or whether they are single or double stranded or whether…. I would like to know all of that. So is that what you'd like to talk about next?

Vincent: I am happy to talk about that.

Dick: Okay.

Vincent: How do you classify viruses? Actually this is a very good point. You know why it's a great point, because we probably throw virus names around and people don't know what we are talking about, right.

Dick: Exactly.

Vincent: So, do I have a lecture on virus families? Let's see.

Dick: Why are they called families and not, you know, the normal classification of life as Linnaeus originally conceived it and then was expanded upon by biology? We don't classify viruses this way because there are no species, is that what you are saying?

Vincent: Well, so we use the classical Linnaean hierarchical system, what does that mean Dick? You would know better than I.

Dick: Well it's a binomial designation.

Vincent: What does binomial mean, two things?

Dick: It has a genus and a species name, a genus and a species.

[0:02:00]

Vincent: So, there's a kingdom.

Dick: Okay.

Vincent: Not for viruses though.

Dick: No.

Vincent: Did you know how many kingdoms there are? Plants, animals, bacteria, archaea, what's the other?

Dick: There are five over all. Five.

Vincent: You don't know what they are though?

Dick: Ah, there are prokaryotes, eukaryotes, ah….

Vincent: No, there are more prokaryotes than eukaryotes.

Dick: There is a book called *The Five Kingdoms*, it is written by Lynn Margulis or Mar-co-las as she pronounces it. And the five kingdoms are Protista and….

Vincent: Six kingdoms.

Dick: Six kingdoms, we are up to six now.

Vincent: It has now been revised.

Dick: Okay.

Vincent: You ready?

Dick: Yep.

Vincent: Animals, Plants, Fungi, Proteus, Archaea, and Bacteria.

Dick: Right. What happened to the viruses?

Vincent: Some text books only have five. No they don't have a… they are not living. They are not in the tree of life. But we are using a similar….

Dick: That raises another question doesn't it?

Vincent: So in the classical classification system, if you will, the Linnaean, you have kingdom, phylum, class, order. So we don't use any of those.

Dick: Then family, genus, species.

Vincent: Yes, then family, genus, species are what we use for viruses. So we start with families which are the words ending in *ae*— *Picornaviridae*, *Orthomyxoviridae*. Okay, when you would use that family name you would italicize it. Or you can say, picornavirus in which case you don't, you just refer to it. So that's the family.

Then we have genera.

Dick: Right.

Vincent: Like enteroviruses, hepatoviruses within a family. Let's do a better job. We have enteroviruses, rhinoviruses, and cardioviruses within the *Picornaviridae*. And then we have species within the genera. Poliovirus type 1 is a species.

Dick: So is every virus particle a different species?

Vincent: No.

Dick: I don't mean a….

Vincent: You know, that's a good question because it's a quasi-species as you know.

Dick: Right.

Vincent: You know many virologists don't buy this species concept.

Dick: I'm sure.

Vincent: In fact you know the plant virologists don't use any of this. They don't believe in this classification, they just give them virus names.

Dick: Right.

[0:04:04]

Vincent: But the animal viruses they tend to fall into family and genus. The species, you know, the classical definition of a species is two organisms that don't reproduce, right.

Dick: Right, or at least they are genetically separate.

Vincent: In viruses it's a very messy definition.

Dick: It's messy. It gets messy at some level because there are lots of exceptions to all the rules.

Vincent: Well that's why we make rules right?

Dick: I think so. The moment you make one it's broken.

Vincent: Species….

Dick: Don't even go there.

Vincent: There are many definitions of species. A common definition is a group of organisms capable of interbreeding and producing fertile offspring.

Dick: Right.

Vincent: And separating from others such group…. So virus… for viruses um….

Dick: They breed true obviously.

Vincent: Polio Virus Type I can all but you know polio virus Type I and II don't… they do recombine actually.

Dick: Okay.

Vincent: So they were called polio type 1 and type 2 for other reasons, not for species reasons. I don't really like species for viruses. Family and genus is good for classifying. So how did we do this?

Well years ago you would, before we had any molecular biology, we did classification of viruses and we classified them on a number of characteristics other than what we do today. We used to do the nature of the nucleic acid.

Dick: Right.

Vincent: The symmetry of the shell.

Dick: Okay.

Vincent: You know capsid, envelope, etcetera.

Dick: Yep.

Vincent: Whether or not it had a lipid membrane, and the dimensions.

Dick: Hum.

Vincent: So various sizes or if you were beyond a certain size you are usually not in the same family basically.

Dick: What about host characteristics?

Vincent: Host characteristics, disease, tropism—absolutely—antigenic character.

Dick: Right.

Vincent: But nowadays it's mainly done by sequence because it turns out the sequence really tells you everything and you can put viruses in families and genera just by the sequence.

[0:06:05]

Dick: Well Vince let's go back to one of our earlier sessions where I was asked to give a little description of West Nile Virus as an example of a single virus species, right, the West Nile virus. There are 23 known isolates, all different. They differ by their genomes.

Vincent: Isolates.

Dick: Right, so….

Vincent: Now we have hundreds of swine flu H1N1 2009 isolates.

Dick: Of course. So what would you call each of the… you call them isolates of course.

Vincent: Isolates, absolutely.

Dick: Sub-species.

Vincent: No, not even sub-species, just isolates because it's the same species you are just getting different isolates.

Dick: But they are geographically restricted apparently. So that's how come we knew that the West Nile virus that we are dealing with in the United States came from the Middle East and not from, let's say the south of France. Ah, they stay put.

Vincent: No, that's not always the fault of the virus.

Dick: No, I wasn't saying it at all. It might be the fault of the mosquito or the host, the mammalian host or something else, right.

Vincent: Sure, the weather, who knows.

Dick: But isn't it interesting though because this virus originated in the West Nile District of Uganda.

Vincent: Right.

Dick: And it spread throughout Europe and Asia, and even in this country now it's become different by natural selection.

Vincent: Well that makes sense right?

Dick: It does to me.

Vincent: Because everything is different here than it is in the West Nile District.

Dick: So the host influences.

Vincent: Of course.

Dick: Not just the virus structure but the virulence as well because the virulence in this country has gone down over the initial input.

Vincent: Are you sure?

Dick: We had… the death rate from West Nile virus in the beginning I think was over 8% and it's now below 2%. I know what you're going to say.

Vincent: Isn't that because there is some immunity that's protective?

Dick: Or because we recognize it earlier and we treat them sooner, it's possible for that too but….

Vincent: Is it… has it… have experiments been done where the effect of the different isolate sequences on virulence has been studied, probably not.

[0:08:05]

Dick: Oh no, no, no. I think it has been studied. In fact this guy Vincent Deubel in the Pasteur Institute in France has done those studies because he's got all 23 isolates, now 24 from the United States. That's the hope that this virus will attenuate as it serially transferred from host to host so the crows won't all die off from this thing eventually.

Vincent: What does the virus care about crows?

Dick: Well that's….

Vincent: What do crows have to do with people? And what does this have to do with classification?

Dick: I think it has something to do with understanding how viruses change overtime because what you're suggesting as from this classification at least at some point there's no change, that is its general shape, its general characteristics, but there's always change going on as we've talked before about influenza virus, for instance. There is always change going on, right? So it still maintains it general characteristics and yet it remains….

Vincent: **Dick** a DNA virus remains a DNA virus.

Dick: Right.

Vincent: You have a capsid, you can always have a capsid; you are not going to acquire an envelope.

Dick: That exactly right, but the sequence of DNA changes.

Vincent: The sequence changes and how much that's expressed into protein depend on the flexibility of the virus and its structure and its proteins and so forth. So flu happens to be very plastic, its glycoproteins can handle an enormous amount of amino acid changes. Other viruses are much less. Measles has… makes a lot of errors in its RNA, genome, but those are not found in the proteins because they are lethal.

Dick: Ah, I see.

Vincent: Measles is not as plastic as influenza. We don't understand why.

Dick: Rabies is not so plastic either.

Vincent: Yeah, it doesn't vary all that much. So this classification now you can look at the sequence and say hmm, this is a polio virus, no doubt about it.

And interestingly, so let me give you an example of a classification.

Dick: Please.

Vincent: The family picornaviridae or the picornavirus family. A genus is the enterovirus and one of the species is poliovirus.

Dick: Okay.

[0:10:00]

Vincent: Another species is coxsacki virus. Polio viruses and coxsacki viruses, if you co-infect cells, [they] will not recombine.

Dick: What about rotavirus?

Vincent: However, there are some examples of naturally occurring recombinants which seem to have sequence from polio and coxsacki and you can create such recombinants in the laboratory.

Dick: Wow.

Vincent: So the classic definition of species is not really applicable. Another genus within the picornaviridae is rhinovirus.

Dick: Uh huh.

Vincent: Now the organization that handles classification of viruses is the International Committee on the Taxonomy of Viruses, the ICTV. Every year they have a meeting and they review all the new

proposals. The new proposal is that the enterovirus and rhinovirus genera are going to be combined because sequence analysis shows they should be the same genus.

What did you say about rotavirus? It's a different family.

Dick: Just asked you if it was in there or not because it's a….

Vincent: It's a reovirus family, reoviridae.

Dick: But it's an enterovirus too though.

Vincent: It is an enteric virus but, and you know what it's also, icosahedral, but it has a very different characteristics. It has ten to twelve pieces of double-stranded RNA. It has a double-shell capsid.

Dick: Wow.

Vincent: It's much bigger, totally different. So that's why it's in a different family.

Dick: Now, the sixty-four thousand million dollar question. As of right now, this very moment, as a human being, how many viruses will infect me of all the ones you know about? How many different viruses infect humans?

Vincent: Well the first question is how many different viruses do we know about?

Dick: Okay, that would be my first question. How many—and you're talking about animal viruses now correct? Yes, because plant viruses are over here someplace.

Vincent: No we can…

Dick: Do you want to throw in plant viruses also?

Vincent: In terms of the term… the total number of viruses?

Dick: The viruses ever discovered.

Vincent: Let's see, I've got that number here.

Dick: Oh it's big. It's a big number.

[0:12:00]

Vincent: Okay, so far we have 40,000 virus isolates; this is as of 2008 from bacteria, plants, and animals and 73 families, 287 genera, and 1,950 species.

Dick: Wow.

Vincent: But remember….

Dick: How many altogether, only 1,550?

Vincent: One-thousand nine-hundred and fifty [1,950] species, identified though, isolated.

Dick: Sure.

Vincent: Remember there are a million virions per mil of sea water and most of them unidentified.

Dick: That's what I was going to say.

Vincent: How many infect you? I don't know the number. We could count it, but I would say no more than a few hundred known viruses. You know, we've got the picornas, the paramyxos, the influenzas, the rhabdos, the herpes, the pox viruses and I'm missing many more, polyomas. But I am sure there are many more that we haven't discovered.

Dick: Right. Yeah they emerge regularly from the wildlife that we keep disturbing.

Vincent: So the new arenavirus from Africa that we talked about last time just emerged, never seen it before, but it was there, I can guarantee you it's been in some animal in the forest in Africa for some time and there will be many more.

It's all a matter of what we look for. This is the age of virus discovery as you know. We have great techniques for detecting and sequencing new viruses and if you look you will find.

Dick: In fact there was a TED lecture on that that I was at.

Vincent: Joe DeRisi.

Dick: That's right.

Vincent: And of course, our colleague here, Ian Lipkin, you know is also a virus pirate. Is that the right word or virus hunter?

Dick: Virus hunter.

Vincent: And they are training the next generation of course. In fact out of Joe DeRisi's lab is David Wang who is at Washington University in St. Louis and he's a virus hunter.

Dick: Yep. How do you hunt for virus Vince?

Vincent: You just sequence nowadays. It used to be we did microarrays. You know what a microarray is?

Dick: I do of course.

Vincent: Do our listeners know?

Dick: I think we should tell them.

Vincent: You take a class microscope slide and spot with a robot hundreds and hundreds of small sequences from whatever you're looking for. So if you want you could put a sequence of every virus on this chip. It's called a chip but it's a glass slide.

[0:14:07]

Dick: Yep.

Vincent: And then you can incubate that with a sample.

Dick: Hybridize.

Vincent: Hybridize.

Dick: Yep.

Vincent: But we don't do that anymore.

Dick: What's the reporter molecule like? I mean how does that actually work?

Vincent: So what you would do is let's say I take a little snot from you and I extract RNA because I want to find a virus. I would then turn that RNA into DNA, and the nucleotides I used would be labeled with a florescent dye, either a green one or a red dye.

Dick: Okay.

Vincent: And that would be hybridized with this microarray chip and then spread by a florescent reader.

Dick: Got it.

Vincent: This is a beautiful technology but you're limited by the sequences you put on the chip, right.

Dick: Of course.

Vincent: So you are limited to known sequences.

Dick: Right.

Vincent: Now what we just do is take that snot, extract the RNA, convert it to DNA and sequence it.

Dick: Ah, easier to do it.

Vincent: There's a new technique called pyrosequencing.

Dick: Pyro implies like you are going to burn something.

[0:15:00]

Vincent: It does, but it has to do with pyrophosphate not pyro in the burning sense because of the chemistry that's involved is pyrophosphate.

Dick: Oh, okay.

Vincent: Started by a company called 454 which is up in Branston, what's the town in Connecticut? Branford, Connecticut.

Dick: Branford.

Vincent: I'll tell you the story of 454. The man who developed this technology, he wanted to sequence his daughter's genome, she had some genetic disease and he wanted to know so he thought of this sequencing technology and then the company has since been bought by Roche.

Now they can sequence 600 million bases for about $5,000.

Dick: Wow.

Vincent: So you give them a sample and they'll give you a half a million bases, 500 million bases for five grand. The cost is coming down. As you know it will probably be a thousand dollars pretty soon.

Dick: That's amazing.

Vincent: So I take your snot, convert it to DNA and send it to 454 and sequence it. You throw away… now this is why we have bioinformaticians involved, you have to pull out all the host sequences that you are going to get and all the known genes and so forth, and you get a hundred bases of some virus that's in you. And that's how you do it.

And Universities now are purchasing these machines so you don't have to send them to the company. We have one here at Columbia.

[0:16:20]

Dick: Nice.

Vincent: And that's how you discover viruses these days. So Dick I would say you and I go off, we'll trap some animals, get some RNA and sequence it. Just see… get out your credit card, five grand.

Dick: Not a problem. I just signed a contract with a book company, I can do this.

Vincent: And then you get a new virus, you can write a grant to study it.

Dick: I can name it after myself.

Vincent: But you wouldn't do that.

Dick: I would not. I would name it after you Vince.

Vincent: So this 40,000 isolates….

Dick: I want the cure named after me.

Vincent: There are, you know, if you took all the viruses in the oceans and put them end-to-end it would go two hundred light years into space.

Dick: You what that sounds like, like a Dorothy Parker statement.

Vincent: Doesn't sound like a Carl Sagan? So anyway we are going to discover many, many, many, many new viruses in the future. So that's taxonomy. Taxonomy nowadays is driven by sequencing.

Dick: Got it.

Vincent: In the old days it was driven by phenotype. Today it is driven by sequencing.

Dick: Wow.

Vincent: So you're right, this was a good second thing to talk about. Do you have any questions about classification of viruses, Dick?

Dick: No, now that we know it's not a life form and we can't treat them as species, we can just treat them as entities that we can identify by sequence.

The other question, the big question I would have at this point then is, how different does a virus isolate have to be to be considered unique?

Vincent: You want a number?

Dick: Yeah, if you have one. How many base pairs different does it have to be? What is the… in other words what I am really asking is what is the error rate for sequencing versus the actual difference between one isolate and another?

Vincent: Nah, well the, this is a problem because the sequencing error rate is quite low but the amplification when you take RNA and you amplify it by polymerase chain reaction, there you have error.

[0:18:04]

Dick: Yep.

Vincent: And so that makes it difficult to look at low-level variation.

Dick: You do repeats then, yes?

Vincent: Well then you're going to get errors somewhere else.

Dick: True.

Vincent: And then you don't know which one is real.

Dick: So how many of the real sequences that we have are actual real sequences?

Vincent: See Dick the problem is they're all sequences of the majority population.

Dick: Right.

Vincent: See what I'm… Viruses are quasi-species. We haven't talked about this but it isn't one sequence. When you isolate a virus it's not just one sequence, it's a mixture.

Dick: Of course.

Vincent: Every virus particle has a difference sequence. But when you sequence the population you get a consensus.

Dick: Got it.

Vincent: And so it doesn't reflect the diversity of the population. Now with these deep sequencing techniques as they're known as, we have the potential to take a virus stock and sequence and learn all the different variants in that stock.

Dick: Got it.

Vincent: But you are going to be limited by the error rate.

Dick: Right.

Vincent: Of the amplification step.

Dick: Yeah, which is what?

Vincent: They are probably a ways…. You know the most faithful polymerase is… I don't want to make a statement here, let's look it up.

Dick: He doesn't want to get a letter like I get. I get all the letters; he doesn't get any of them. That's not fair.

Vincent: I don't mind. So you know the most error prone PCR, thermo enzymes that are used in PCR make an error about every thousand bases.

Dick: Every thousand bases.

Vincent: But there are high-fidelity polymerases that have much less error rate.

Dick: So wouldn't you have to go back after you amplify and got your virus isolate and were pretty sure of what it was, you could go back with the polymerase that doesn't make so many errors and get the actual sequence?

Vincent: Well if you are just sequencing a viral genome you can do that. But if you want to know the variation in a stock it's harder.

Dick: Alright, right.

Vincent: Let's see what the error rate is. So there are high-fidelity enzymes that you can use.

Dick: So do we really know Greg Venter's sequence or are we just looking at an approximation?

[0:20:02]

Vincent: Oh well they do what's called… each position is done many times, it's called the coverage.

Dick: Okay.

Vincent: So for a genome like us, human or mammoth genome, they want 50 to 60 times coverage at each position otherwise you sequence each base 50 or 60 times.

Dick: I see. Wow.

Vincent: So a mammoth genome sequence was recently published. I don't know if you saw this.

Dick: Yes, I did actually. I was very interesting.

Vincent: And they had a… there DNA was very degraded because it was old. It was 20,000 years old but they got six-fold coverage at each position but they want to get 60 to have more confidence.

Dick: Right.

Vincent: And the other problem with that sequencing project was that the runs were very short, one or two hundred bases. So it's hard to stitch it together. So they used the elephant genome as a template to align it. It turns out that the mammoth genome was only 2% different from elephants.

Dick: Amazing.

Vincent: So there was not much pressure going from mammoths to elephants. Whereas going from chimps to humans was more like 5%. You know we had to get up on two legs to escape the tigers and lions that wanted to eat the chimps, right.

Dick: It's true. Yep.

Vincent: That was the selection pressure.

Alright PCR error rate—we have to get an answer because I will not….

Dick: Sleep tonight until you know.

Vincent: I can't record this without an error. Okay here we go. So it's one in a million instead of one in a thousand.

Dick: That's pretty good.

Vincent: So there are different sources of these enzymes.

Dick: Right.

Vincent: Pyrococcus furiosus and then there are enzymes to….

Dick: Is that an extremophile?

Vincent: Yeah, they are from the vents.

Dick: Okay.

Vincent: Bottom of the ocean, very hot. And so these enzymes are put through thermal cycling and so they have to be thermal resistant.

Dick: Sure.

Vincent: So you go from about 1 in 1,000 to 1 in 100,000 to 1 in 1,000,000. Those are the good ones, the one in a million errors. But in a 10kb virus genome it's not bad but in eukaryotic, big host genomes like ours, it can be a problem.

[0:21:59]

Dick: Right. So now we have the structure. We have classification. So now we have to start talking about the process of infection.

Vincent: Yeah.

[0:22:25] BREAK START

[0:23:27] BREAK END

Vincent: Today there are two emails that I want to use to discuss the swine flu immunizations of 1976. The first one is from Gus, and Gus writes:

"Hello Professor Racaniello, first I want to say that I love your podcast. I am fascinated by the topic and may have majored in virology if I was exposed to this material earlier in life. I am a chemistry major and have worked in the semiconductor manufacturing field for more than 20 years building integrated circuits and now work for a small company in Monroe, Washington making state-of-the-art compact, high-powered solid state laser systems.

"I'm glad you've decided to devote part of the show to basic virology, so called *Virology 101*. Although I understand most of the material on the show, some of the common virology terms used by you and Dick

are foreign to me. In future virology primers it would be helpful to define and explain some of the more common virology terms commonly used in the field which may not be so common to us lay people.

“For instance, I’ve heard the term negative strand RNA mentioned a few times on the podcast. It would be nice to explain exactly what a negative strand of RNA virus is and how it works. I googled the term but the definition I found online is fuzzy.

“The negative strand virus was explained as the antisense strand of RNA which does not encode for mRNA, mRNA encodes proteins. What does the antisense strand of RNA encode for and how does it work?”

Okay, let’s take that first before we move on to his last question.

Negative strand is the opposite of the plus strand and this is with reference to RNA. Plus strand RNA is the RNA that is used to encode protein. So in the cell mRNA is positive polarity, or plus strand, and that is the RNA that is translated on ribosomes to make proteins. Many RNA viruses have plus strand RNA genomes, like the picornaviruses, of which polio viruses and rhino viruses are members.

So plus strand RNA viruses have an RNA in there virion that can be directly translated when it enters the cell. The plus strand RNA is recognized by the cell and is translated.

[0:26:00]

Now as you know there are many viruses that have negative strand RNA genomes. And influenza virus is an example of one of those. A negative strand is the opposite of a plus strand. It cannot be translated by the cells translation apparatus. Okay, so in order to make protein from influenza virus genomes, the negative strand RNA first has to be copied to a positive strand.

So the influenza virus RNA, which is negative stranded, enters the cell, is transported into the nucleus where it is copied to a plus strand RNA, and then that goes back out to the cytoplasm and is translated by ribosomes. So the plus strand copy of the influenza virus negative strand RNA can be translated.

So this brings up an interesting issue. Viruses with plus strand RNA genomes don’t need to carry any enzymes into the cell in order to copy their genomes because their RNAs can be recognized directly by the translation apparatus of the cell. In contrast, viruses with negative strand genomes, like influenza virus, have to carry and enzyme into the cell which will copy the negative strands into plus strands and that’s because the cell doesn’t have enzymes that can copy RNAs such as viral RNAs. The viral RNA of influenza virus, the negative strand RNA, cannot be copied by the cell into a plus strand so the virus has to carry in an enzyme.

So all negative strand RNA viruses have to carry in an enzyme, an RNA polymerase, to copy their negative strand RNAs into plus strands that can be translated into protein. Plus strand RNAs don’t have to do that.

[0:27:56]

So I hope that's clear. We'll certainly go over this in the segment of *Virology 101* which deals with replication of genomes of viruses. It is an important concept. I understand we've mentioned it a lot. So that's what it means. Negative strand, the opposite of plus strand, cannot be translated.

Alright so Gus' second part, "Here's one more last item. I will post a link to a suppressed *60 Minutes* episode back from 1977-78 which covered a swine flu vaccination campaign that seemed to go wrong. I'd really like to hear your perspective on this *60 Minutes* episode."

So he gave us a link to an episode of *60 Minutes* which explored the adverse effects associated with the swine flu campaign.

"Thanks much. P.S. Does Dick have a Twitter account? I follow both of you and Alan on Twitter but haven't been able to find Dick's account if he has one."

No, I'm sorry Dick doesn't have a Twitter account. He doesn't, he simply is not interested in doing all of these online and social media type things. So the only way you can contact him is by emailing TWiV at TWiV.tv.

Okay, now Swiss Compass wrote, "Regarding the probably impending swine flu vaccinations and concerns over Guillain-Barre syndrome or similar fears, could you please consider discussing this issue a bit more deeply? There is a growing resistance to accepting vaccinations and a lot of confusion on blogs and websites. A recent argument against accepting vaccinations is a *60 Minutes* report from 1977-78 on swine flu."

And he sent the YouTube link to the same video that Gus had sent, the *60 Minutes* episode from 1977 on swine flu and the adverse effects.

[0:30:00]

"Please consider discussing this issue on TWiV, and possibly laying out simple facts that can be used to dispel the fear, uncertainty, and doubt. Also, is the National Vaccine Information Center a credible organization?"

The National Vaccine Information Center is an organization which is aimed at allowing the public to make their own decisions about vaccinations. As far as I can tell there are no virologists associated with it so I don't know how they can present a balanced view about viral vaccines. So I don't want to make a statement about whether it's credible or not, but in my view it doesn't really present all the information that you need to make a decision.

What about swine flu immunizations and Guillain-Barre? So let's go back to 1976 and the outbreak of swine flu at Fort Dix, which is a military fort in New Jersey. So in January of 1976 there was an outbreak of fibril respiratory disease. There are 19,000 people at Fort Dix at the time and virological studies showed that it was a swine influenza virus, and it was called A/New Jersey/76 Hsw1N1. So the hemagglutinin at the time was called Hsw1 because it was a direct descendant of the hemagglutinin of the influenza virus that infected pigs starting in 1918.

Now at Fort Dix in that year, in January '76, the virus appeared to infect about 230 solders, caused severe disease in 13 and one recruit died. Now at the time we thought that the 1918 pandemic of influenza was caused by a swine virus. We didn't have the virus that we have today, the rescued 1918 virus which shows that it's most likely an avian virus. So we thought it was a swine virus and the isolation of swine influenza virus from recruits in 1976 scared many people. So many scientists were concerned that the virus of 1918 had come back to Fort Dix and would soon cause a catastrophic outbreak.

Now in 1976 I was a Ph.D. student at Mount Sinai School of Medicine in New York and in the Department of Microbiology and the chair of that department was Dr. Edwin Kilbourne who was a very well-known influenza researcher. And he in fact was responsible every year for producing the high yielding influenza recombinants which were used to produce influenza vaccines. Now he and others felt that it was important to immunize the population against this virus so they convinced basically the U.S. Public Health Service to contract for producing a 150 million doses of vaccine against the Hsw1N1 virus.

In March of that year, President Ford announced a program to inoculate everyone in the U.S. against swine flu. And those of us who remember, he went on television and got the shot on TV. In October the immunizations began. The whole program was stopped in December, only about 45 million doses were distributed by that time because it was clear that the virus never went outside of Fort Dix.

In retrospect we probably should have stock piled the vaccine and waited to see whether it would spread. Now remember, first outbreak was January, the flu season is over at the end of May, so we don't see flu typically over the summer, so the idea was that it would come back in the fall. That's why we started immunizing in October.

But in fact it should have spread, if it was going to be a pandemic strain it should started spreading in January and February, but it did not. There was no evidence for a spread outside of Fort Dix. Now why?

Well the fort is quite isolated and there's limited contact between basic trainees and others who travel outside the facility. And the older people at the facility may have been immune because there were some vaccines given to military personnel from 1955 to 1969 that had a swine influenza component to them.

The other issues this most likely wasn't the right virus to circulate or to be transmitted among people. It was probably a accidental infection from a pig and there was limited circulation because in contrast to the 2009 origin H1N1 virus, it just didn't have the right collection of genes to allow it to spread efficiently in people.

Today of course we know that the 1918 influenza virus was probably derived from an avian virus not a swine strain. And if we knew that in 1976 we never would have carried out this immunization campaign.

Now getting back to how this virus got into the fort, into Fort Dix, we really don't know. We don't know the origin of A/New Jersey/76. Of course one theory is that the swine virus was brought to the fort early in 1976, in January the recruits were returning from the holidays so some of them might have had

contact with pigs and brought the virus in in January, but none of them who were interviewed admitted to having any contact with pigs. So we really don't know the origin of that virus. It circulated for about a month at Fort Dix and then disappeared and that was the end of it.

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So really in retrospect we should not have started this immunization campaign, but of course hind sight is always 20-20. We've certainly learned a lot from that experience.

Now one of the unfortunate consequences of the 45 million people who got the vaccine, there were about 500 cases of a syndrome known as Guillain-Barre, G U I L L A I N - B A R R E. This is basically an autoimmune disease. The body's immune system attacks the peripheral nervous system. And we haven't talked much about autoimmune diseases on TWiV but basically you make an immune response to foreign antigens but sometimes you make immune responses against yourself and that's problematic and you have disease. We'll probably talk about that in one of our *Virology 101* episodes dealing with pathogenesis.

Now the symptoms of this disorder are varied. They include limb weakness, tingling sensations in the legs, so that the illness begins in the legs, you can have a partial paralysis and that can move up the body to the trunk and eventually to the arms and upper body. When it does reach the upper body its considered life threatening because the respiratory muscles can be compromised and you have to be put on a respirator.

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Most people recover from Guillain-Barre, but about 5-6% of them may die. So there were a few deaths from… associated with the Guillain-Barre cases, the 500 which occurred, again after being temporally associated with the swine flu immunization of 1976, the fall of 1976.

So Guillain-Barre is a syndrome because we don't know what causes it. We don't have a specific disease causing agent. It's a collection of symptoms so we call that a syndrome. It has been associated not only with the 1976 swine flu vaccination program but with other infectious diseases and with bacteria, in particular with a bacteria called campylobacter jejuni, which is another common infection that triggers Guillain-Barre syndrome. And there are other respiratory and intestinal illnesses that are associated with Guillain-Barre.

The 1976 swine flu vaccine is really the only influenza vaccine that has had significant association with Guillain-Barre syndrome. We don't know why this is the case. A number of theories have been set forth as to why. And one of them was that the vaccine may have been contaminated with this bacteria, campylobacter, but that's not true, that's been disproven. Another was that the vaccine somehow was contaminated with material that induced antibodies against neurological tissues but that hypothesis has not been supported by any experimental results. So we really don't know why that particular vaccine induced Guillain-Barre syndrome.

So remember it was given to 45 million people and each year more people than that are given seasonal influenza vaccines and there is a lower association with Guillain-Barre in those.

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So the real question is whether the vaccine against the 2009 H1N1 swine-origin influenza virus, the current pandemic strain is going to be associated with Guillain-Barre? Of course there is no way to predict this at all. Remember these vaccines are produced by making a recombinant or a re-assortment between the strain—in this case the 2009 H1N1—and a high-yielding virus which grows well in eggs. The re-assorted will have the surface glycoproteins, the hemagglutinin and the neuraminidase of the 2009 H1N1 virus and other genes from this high-yielding strain.

Now the 1976 vaccine also was an H1N1 but it was quite different and most of the other proteins of the virus were derived from the high-yielding virus. So the only difference would be the surface glycoproteins between the 1976 and the 2009 H1N1 vaccines. So I see no reason why the hemagglutinin or the neuraminidase would induce Guillain-Barre on their own. It could be a contaminate, but as I said that's never been proven. And the hemagglutinin and neuraminidase of the 2009 virus are quite different from that of 1976.

So you know in the end you have to balance the side effects or the potential side effects of a vaccine with the seriousness of the disease. We still don't have a good handle on the precise mortality, or the case mortality ratio of this new strain. That's not something we'll have for a while because we have to do some statistics to figure that out. The current numbers look like it's about 0.4 percent, which is certainly higher than the incidence of Guillain-Barre.

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But I will be getting the swine-origin vaccine in the fall of 2009. I will take my chance because I don't want to get influenza. I see no reason to think that this vaccine will be associated with Guillain-Barre to the extent the 1976 vaccine was.

Now the *60 Minutes* episode, if you have a look at it that was submitted to us by the two listeners, deals with the whole 1976 controversy. First the issue of why we were immunized at all, because it didn't look like this virus was spreading. And of course if you compare the spread of the 2009 virus with that of the 1976 swine-origin virus they are quite different. It was quite clear very quickly after the first isolates of the 2009 virus that it was going to spread globally. And we had no such indication in 1976 for many months. So again, in retrospect, it was clear that the virus was going nowhere. So we probably shouldn't have used it but it was approved by scientists, by virologists, by the Public Health Service and that's a decision we have to live with.

Also the *60 Minutes* episode focuses on the unfortunate aspect, of course, and that is that a number of people contracted Guillain-Barre and some of them had permanent paralysis. So we went from being healthy to being disabled as a consequence of receiving that vaccine, which in the end wasn't necessary at all. So that's extremely unfortunate but I see no reason why the same events will unfold with the

current vaccine. I see no scientific reason to believe that the new vaccine will have similar effects. So that's all I can tell you about the 1976 swine immunization campaign and Guillain-Barre.

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I'll post a few links to articles in the show notes and if you have questions, of course do send them to TWiV at TWiV.tv and we'll get to them as soon as we can.

I thought you'd like to hear that sooner rather than later so that's why I've made this little discussion on my own.

I do want to close with some science picks of the week, and you may have heard Dick mentioning a book earlier called *The Five Kingdoms: An Illustrated Guide to the Phyla of Life on Earth*. And that's by Lynn Margulis and Karlene Schwartz. It's basically a review of all the phyla from bacteria all the way up to higher organisms. So if our discussion of the kingdoms and phyla confused you, you might want to have a look at that. It's apparently a very good book.

My pick is book called *A Genetic Switch* by Mark Ptashne. This was a 1986 book from a virologist who worked on bacterial viruses and used them, in particular bacteria phage lambda, to understand how genes are regulated. It's a classic. It's very well written, very clearly written. And I think you'll enjoy it. A little paperback book that's very accessible.

Of course if you are interested in other good science podcasts check out sciencepodcasters.org and promednetwork.com where you can find other great science podcasts.

Remember that TWiV has a web site where we post show notes, TWiV.tv. Do send us your questions. As usual, tell us what you think, what you'd like to know, what we got wrong, TWiV@twiv.tv.

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You can also send us an mp3 file if you want or you can call us on Skype, our user name is TWiVPodcast, all one word, and you have about ten minutes to leave a voice mail.

You have been listening to *This Week in Virology*, the podcast all about viruses. Thanks for listening, we'll be back next week. Another TWiV is viral.

[0:46:29] END

Transcribed by Steve Stokowski.