Bogoch Replikins Pandemic Prevention: Increase of Strain-Specific Influenza Genomic Replikin Counts™ , Having Predicted Outbreaks and their Location Seven Times Consecutively, Up to Two Years in Advance, Provides Time for Prevention of Pandemics

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Figure 1- Replikin Count™ Increases in H1N1 and H5N1 Globally and Locally

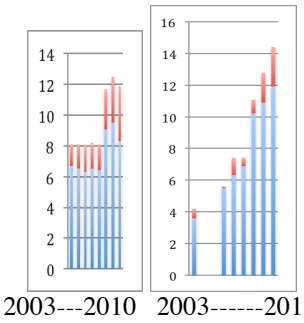
Annual Mean Replikin Counts™, blue; Standard Deviation of the Mean (SD), red

Predictions: **H1N1** Sixth and Seventh Predictions (3,10) **H5N1** Fifth Prediction (4)

H1N1 Hemagglutinin gene
All of the sequences listed on Pubmed

Global Replikin Count

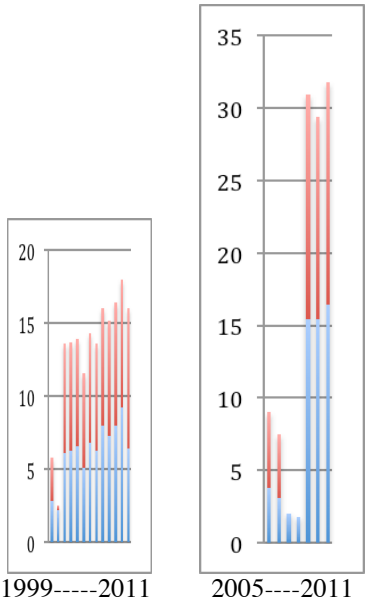
Mexico Replikin Count



H5N1 pB1 gene
All of the sequences listed on Pubmed

Global Replikin Count

Cambodia Replikin Count



Outbreaks: **H1N1 2009, 2010, 2011, 2012 in Mexico** (5-7,10)

H5N1 2011, 2012 in Cambodia (8,15)

Legend for Figure 1: Note for H1N1 the gradual increase in the annual mean Replikin Count™ globally; and in contrast, the approximately 40% increase in the H1N1 Count in Mexico between 2008 and 2009 which preceded the selective H1N1 outbreak in Mexico in 2009 and the pandemic. Similarly, note the gradual increase in the global mean H5N1 Counts and the marked increase in the H5N1 p B1 Count in Cambodia in 2009 (pB1-F2 frame Count alone increased from 17.8 to 28.9, not shown) which was followed by the unique outbreaks in Cambodia in 2011 and 2012 (now also being reported in neighboring Vietnam). The size of the SD (in red) indicates the proportion of viruses in a given population with high Replikin Counts™ (previously associated with rapid replication (1,12)) and the diversity of the Replikin Counts™ (number of Replikins per 100 amino acids) in the genomes of a virus population (1). In Figure 1, there is a greater proportion of the H5N1 virus population than that of H1N1 engaged in rapid replication. During low rate of replication 'rest periods' and small values of the mean Count (in blue), the SD (in red) is small or zero, as also observed with influenza B over many decades (1); during periods of rapid replication, the SD is larger in relation to the mean. Global Counts are those for all countries combined; Counts for Mexico and Cambodia are those in each country respectively.

Summary

Earlier studies have shown that the increased concentration of a new class of virus genomic peptides, Replikins, precedes and predicts virus outbreaks (1). We now find that the area in the genome of the highest concentration of Replikins, and the country in which this peak exists in scout viruses, have permitted in the past five years seven consecutive accurate predictions of the geographic localization of coming outbreaks, including those now realized in Mexico for H1N1, and in Cambodia for H5N1. Real-time Replikin analysis of the evolution of the virus genome identified both mutations and structural reorganization of the hemagglutinin and p B1 genes over several years before each outbreak. This information, together with the specific Replikin sequences so obtained, permitted solid-phase synthesis of Replikin vaccines in seven days, which blocked H5N1 in chickens (13). The information also now provides up to two years of time to thoroughly test and distribute vaccines to high risk individuals in the countries identified; thus for the first time, a quantitative genomic Replikins method to both predict initial outbreaks and to prevent the development of a pandemic.

Methods

Software based on the authors' algorithm (9) first identified and then counted the replikin peptides in each genomic sequence (Replikin Count™ = number of replikins per 100 amino acids). For each annual group of sequences, the Replikin Count™ mean and standard deviation of the mean (SD) were calculated and compared with other years. Highly statistically significant increases and decreases were examined, for example by strain, host, country, history, year, month or week; by substitution, morbidity, and lethality. The terms 'increase' and 'decrease' of Replikin Counts™ were used only when the p level was less than 0.001. All Counts for H1N1, H5N1 and other influenza strains were each monitored separately, retrospectively and prospectively, from 2003 to 2011 for all countries reporting to Pubmed. Replikin Counts™ were compared to Counts for the same strain in outbreak and non-outbreak ('resting') time periods. Statistical analyses of rate of change, trend, pattern, and growth models in the evolution of each virus strain were initiated. Replikin genes were isolated *in silico* by scanning and identifying those areas of the virus genome which had the highest concentration of replikins. The Count in a given strain was compared with the Count in other strains, and between countries in the same year, and these results compared

with the occurrence of outbreaks, to determine if Count increases could be advance predictive markers of outbreaks of the strain and in the country which were host to those highest Counts (Figure 1). When the eight H5N1 genomic areas were examined year by year, gene areas were found which became upregulated when associated with particular outbreaks. When the upregulation was found to be associated with high infectivity (morbidity over time period), the high Count area was named Replikin Infectivity Gene. When a high Count area in a sequence was found to be associated with high mortality rates, the high Count area was named Replikin Lethality Gene. The Replikin Count™ of these two genes in the H1N1 virus were determined annually from 2001 to 2008 before the pandemic, then during the pandemic 'real-time' every few days, then weekly, from April 2009 to February 2011 (1). Replikin peptides were visualized by two means: a) by linear display of sequences of contiguous numbered amino acids in the primary structure and b) by X-ray diffraction analysis of the 3-dimensional folded structure, which showed the increased coverage by Replikins of the HA gene surface in H1N1 real-time as the Replikin Count™ increased from 3.2 to 5.5 to 10.1 from before to during the course of the pandemic of 2009 (1).

Results

Statistical Analysis of Replikin Count™ Changes

The terms 'increase' and 'decrease' of Replikin Counts™ were used only when the p level for the change was less than 0.001. Additional independent statistical analysis by Associate Professor of Biostatistics Todd MacKenzie, PhD, Dartmouth Medical School showed, for example, that the increase in mean counts for H1N1 globally between 2008 and 2009 of 3.1 (95% Conf. Int. 2.9 to 3.3) was a statistically significant increase: p-value (t-test)=$1/10^{130}$, p-value (Wilcoxon rank sum)<$1/10^{16}$ (1).

First Five Predictions

Figure 1 shows that while the high-lethality H5N1 p B1 gene Replikins Count™ increased gradually globally from 2001 to 2010, uniquely in Cambodia the Count increased markedly in 2009 (Figure 1). Lethal H5N1 outbreaks occurred in Cambodia in 2011 and continue into 2012. This is the fifth instance in which the specific strain and specific location of an influenza outbreak was predicted by the Replikins Count™ (number of genomic Replikin peptides per 100 amino acids) alone, one to two years in advance of the outbreak, together with the country in which the outbreak would occur. The previous four predictions were made for H5N1 in Indonesia in 2006 (11), for H1N1 in Mexico in 2008, which spread to become the global pandemic of 2009 (1), for H1N1 in Europe in 2010 (1), and for H1N1 in Mexico in 2010 for the outbreak there in 2011 (3,8,14). The current Replikins Count™ in Cambodia has not yet decreased, as it does when the outbreaks are over (1). Replikins Count™ warning may enable the prevention of emerging diseases rather than just waiting passively for the outbreak.

Sixth and Seventh Predictions

After the prediction in 2008 of the H1N1 pandemic of 2009 (1,3,7,10), the prediction of the sixth outbreak, again of H1N1 in Mexico, was made in April

2010 (4), (see Figure 1). This prediction was followed by outbreaks in Chihuahua, Mexico in the spring of 2011 (5). Because of the persistent rise in the Replikin Count™ to its highest level in 50 years, the seventh prediction of outbreaks, made in April 2010 (4), again in Mexico, was repeated in August, 2011 (14), and the outbreak was realized in February 2012. The State Health Secretary in Oaxaca, Mexico announced on February 18, 2012 the occurrence of 262 cases of H1N1 influenza with 12 deaths (6). This represents the seventh instance where, for a specific influenza strain, both the outbreak and the country in which the outbreak would occur were predicted by the highest viral genomic Replikin Count™ alone.

Discussion

These are the first accurate predictions of virus outbreaks (1), and now prediction of their location, based on virus genomic changes. The accuracy of Replikin Count™ predictions, acknowledged by the UN FAO (3) and greenlighted by the UK Department of Trade and Investment (16), provides for the first time up to two years of time to respond to specific viral genomic Replikin structures which are in the evolutionary process of preparing for a coming outbreak. The years of mutations and molecular reorganization of genomic Replikins which precede an outbreak, were demonstrated by X-ray diffraction (1). X-ray diffraction analysis of the 3-dimensional folded structure, has shown the increased coverage by Replikins of the HA gene surface in H1N1 real-time as the Replikin Count™ increased from 3.2 to 5.5 to 10.1 over the years during the development and course of the pandemic of 2009 (1). The time taken for these changes to occur, encourages the consideration that prevention of a pandemic is possible for the first time. In contrast, the exact Replikin sequences revealed make it possible to produce in as few as 7 days an effective vaccine for the most recent mutations (13).

If the 2009 H5N1 sequences had been published for example on Pubmed making them available for Replikins analysis in 2009, instead of their apparently first publication in 2011, it would have provided a two-year advance warning of the high mortality human outbreaks now occurring in Cambodia in 2011-2012 (8,15). Two years would have been available to prepare, to test, to distribute, and to administer to high-risk individuals in Cambodia specific Replikins TransFlu™Vaccine or other vaccines. Now that it is recognized by the UN FAO (3) that the Count provides a reliable predictive marker of outbreaks, and seven successive correct predictions have been made in the past five years, genomic sequence analysis should be made available as soon as possible for earliest possible Replikins Count™ Analysis.

The Replikins Count™ is the first quantitative genomic method to predict outbreaks of influenza and other lethal infectious diseases, and thus a quantitative genomic key to explore, track, and predict virus and bacterial evolution. Since 1917, influenza outbreaks invariably follow or accompany marked increases in the Count; and disappearance of the outbreak occurs with decrease in the Count (1). The current data demonstrates that the evolution of a particular strain of

influenza virus, although often gradual over years, with time for host defense adaptation, as for H1N1 in Mexico 2003-2008 (Figure 1), can be rapid, with more immediate disease consequences, as in Indonesia in 2006 (11), and in Cambodia in 2009 for the current outbreak (Figure 1).

A book in preparation, *The Evolution of Lethal Replikins* (12), will provide a fuller report of the evolutionary development of lethal Replikins pathogens in influenza, in other viruses, bacteria and trypanosomes. It will also more fully discuss the role of the Replikins Count™ as a marker of and determinant of lethality.

In 2008, the increasing Replikin Count™ predicted the H1N1 Pandemic of 2009 (3,10). Pharmaceutical companies began their response only when the outbreaks began in 2009, and because of the limitations of the old biological vaccine production methods used, were able to deliver their vaccine to only 20% of those needing it, eight months after the outbreak. Too little, too late.

In 2010-2011, this new Replikins technology again has accurately predicted strain-specific and location-specific outbreaks and thus has made possible the production, testing, and delivery of an updated Replikins vaccine which reflects the most recent Replikin mutations that have occured, - in advance of the next outbreak. Replikins Solid-Phase Synthetic Vaccines can now be further tested to determine if they will offer the following advantages, as was evident in the first trial (13):

1. Key Epitopes: The vaccine is based on parts of the influenza virus proven to be key to clinical outbreaks since 1918, and specifically, the current vaccine for both H1N1 and H5N1 is updated so that it is based on genomic Replikins increasing for the coming outbreaks;
2. Time to outbreak: The advance notice of coming outbreaks, based on Replikin Count™, gives up to two years *before* the outbreak to prepare, test and distribute the vaccine; other vaccines based on biological methods, will require four to eight months *after* the outbreak to be delivered to the population at risk;
3. Time to produce: The vaccine is a completely solid-phase synthetic product which has been manufactured in 7 days;
4. No refrigeration required: The vaccine is delivered freeze-dried.
5. Quantity: The vaccine can be made in unlimited amounts, and at lower costs, for the 7 billion humans and more animals; other vaccines have been limited to approximately 1.25 billion doses delivered 8 months *after* the outbreak, as in 2009;
6. Side effects: No known undesirable contaminants. The solid-phase synthetic vaccine contains no DNA, as proposed for other vaccines) which if incorporated into the human genome, can have unknown generational effects in perpetuity (a potentially very 'long-acting' medication). It contains no contaminating biological substances (because throughout manufacture and delivery the vaccine never is in

contact with any) with potential side effects such as Guillain-Barre Syndrome. For example, Replikins vaccine contain only peptides, which have a short half-life and a long safe history of therapeutic use.

Conclusion

Replikins predictive technology makes possible a practical, relatively inexpensive, country-specified method to undertake the prevention of pandemics.

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