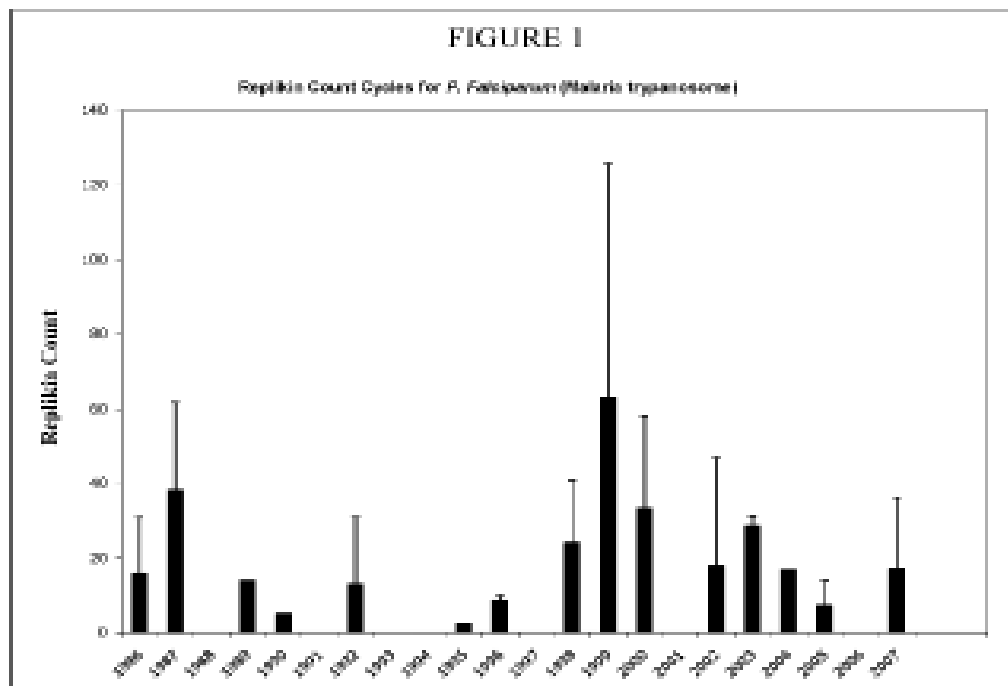


Genome Replikin Count™ Predicts Increased Lethality of Malaria

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REPLIKIN COUNT CYCLES IN MALARIA AND METHODS OF PREDICTING INCREASED MORTALITY



An increase in virulence, morbidity, and/or mortality of a placidium that causes malaria may be predicted by identifying a cycle of Replikin concentration among a plurality of isolates of the species of and identifying a peak in that cycle. An increase in virulence, morbidity, and/or mortality is predicted following the time point or time period when the peak is identified.

This communication is one of four submitted together:

Genome Replikin Count™ Predicts Increased Lethality of Resistant Tuberculosis

Genome Replikin Count™ Predicts Increased Lethality of Malaria

Genome Replikin Count™ Predicts Increased Lethality of Cancer

Genome Replikin Count™ Predicts Increased Infectivity/Lethality of Virus

Replikin Count Cycles Predict Increased Mortality

A cycle of Replikin concentration or “Replikin cycle” of may be seen in Supplement Figure 1. A Replikin cycle is identified by initially isolating at least four isolates or groups of isolates from at least four time points or time periods, for example, an isolate or group of isolates may be obtained in 1999, 2001, 2002, and 2004, or may be obtained in January, May, September, and December of a given year. Isolates may be obtained from more than four time points or time periods and precision of a Replikin Cycle generally will improve with increases in the number of isolates per time point or time period and

with increases in the number of time points or time periods. The Replikin Count of the genome or expressed proteins of each isolate is determined. Replikin Count may be determined in a Replikin Peak Gene, in the entire genome, in a particular gene or gene segment, or in a particular protein or protein fragment of each of the isolates. Mean Replikin Count for a given time point or given time period is determined if a plurality of isolates has been obtained for the given time point or given time period. Replikin Count may then be analyzed per unit time. A cycle in Replikin concentration is identified by four time points or time periods, where the Replikin Count at a second time point or time period is higher than at first time point or time period, the Replikin Count at a third time point or time period is lower than at second time point or time period, and Replikin Count at a fourth time point or time period is higher than at the third time point or time period; or where the Replikin Count at a second time point or time period is lower than at first time point or time period, the Replikin Count at a third time point or time period is higher than at second time point or time period, and Replikin Count at a fourth time point or time period is lower than at the third time point or time period.

A peak in a Replikin cycle is identified within the cycle at a second time point or time period within a Replikin cycle, wherein the Replikin concentration at a first time point or time period immediately preceding the second time point or time period is lower than the Replikin concentration at the second time point or time period, and the Replikin concentration at a third time point or time period immediately following the second time point or time period is lower than the Replikin concentration at the second time point or time period. Replikin peptides of the discovery identified at a peak of the Replikin cycle include Replikin peptides identified at or near the peak of the Replikin cycles including prior to and subsequent to the precise point of the peak. A rising portion of a Replikin cycle is any point at which trend of Replikin concentration in the Replikin cycle is increasing from at least a first time point or time period to at least a second time point or time period and can include a peak. As may be seen in Figures 1-5, an increase in virulence, morbidity, or mortality may be predicted following a rising portion or peak in a Replikin cycle.

Figure 1 illustrates cycling between 1986 and 2007 of annual mean Replikin concentration in the histidine rich protein of *Plasmodium falciparum*. *P. falciparum* is a trypanosome that is most commonly associated with malaria. Two cycles are observable at peaks in 1987 and 1999. A third cycle appears to have begun between 2005 and 2007. Publicly available accession numbers at www.pubmed.com containing amino acid sequence listings for *P. falciparum* were queried using the automated FluForecast[®] software (Replikins, Ltd., Boston, MA). The software analyzed the Replikin Count of each available sequence in Pubmed between 1986 and 2007. The area of the *P. falciparum* genome observed to have the highest concentration of continuous Replikin sequences per 100 amino acids was determined to be the histidine rich protein. The histidine rich proteins include the knob-associated histidine rich protein

Analysis of the mean annual Replikin Count of the histidine rich protein between 1986 and 2007 revealed two cycles of Replikin Count. The first cycle was observed from 1986 to 1995. The second was observed from 1996 to 2005. The peak of the first cycle was

identified in 1987 with a mean annual Replikin Count of 38.2 and standard deviation of ± 23.5 . The peak of the second cycle was identified in 1999 with an even higher mean annual Replikin Count of 62.9 and standard deviation of ± 62.9 (branching and stacking of Replikin sequences within an amino acid sequence generates a Replikin Count of greater than 100 Replikin sequences per 100 amino acids in some sequences). Both the 1987 peak and the 1999 peak were observed to be related to higher human mortality. Following the 1999 peak, mean annual Replikin Counts were observed to fall to a low of 7.4 in 2005 with a standard deviation of ± 6.5 . Mortality rates likewise fell between 2000 and 2005. A third malaria Replikin cycle appears to have begun in 2005 with the observed annual mean Replikin Count increasing from 7.4 ± 6.5 in 2005 to 17.2 ± 19 in 2007. The beginning of the third cycle provides a prediction that Replikin Count may continue to increase along with an increase in malaria mortality rate.

Replikin Count™ Cycling Observed in Other Organisms

The cycling observable for Plasmodium Falciparum in Figure 1 has also been observed in viruses, namely, the H1N1, H2N2, H3N2, H5N1, and H3N8 strains of influenza virus and in West Nile virus. Thus Replikin cycles are observable in both viruses and other organisms. The Replikin concentration of West Nile Virus was earlier found to increase annually through two distinct cycles as the virus expanded in the U.S.: the first from 2000 to 2003, and the second from 2004 to 2007 (p less than 0.001). Increases in the annual number of CDC reported human cases followed each of the virus Replikin concentration increases. Similar correlations also have been shown for Replikin concentrations and human mortality in an influenza H5N1 cycle between 1997 and 2007. The data for Supplement Figure 1 are seen in Table 1 below. Mean annual Replikin Count, standard deviation, significance of annual mean Replikin Count to the lowest annual mean Replikin Count and to the previous annual mean Replikin Count, and number of accession numbers analyzed per annum is provided.

Supplement Table 1- Malaria (Pl Falciparum)

Year	Mean Replikin Count	Standard Deviation	Significance (compared to lowest value)	Significance (compared to previous year)	Number of Accession Records for malaria isolates
1986	15.9	15.2	low>.5		6
1987	38.2	23.5	low<.005	<.02	11
1988					
1989	13.9	0		<.005	1
1990	5.2	0			1
1991					
1992	13	18.2	>.5	<.2	9
1993					
1994					
1995	2.4	0		<.1	1
1996	8.7	1.2	<.01	<.01	3
1997					
1998	24.1	16.7	<.01	<.04	7
1999	62.9	62.9	<.2	<.24	4
2000	33.3	24.7	<.3	<.4	3

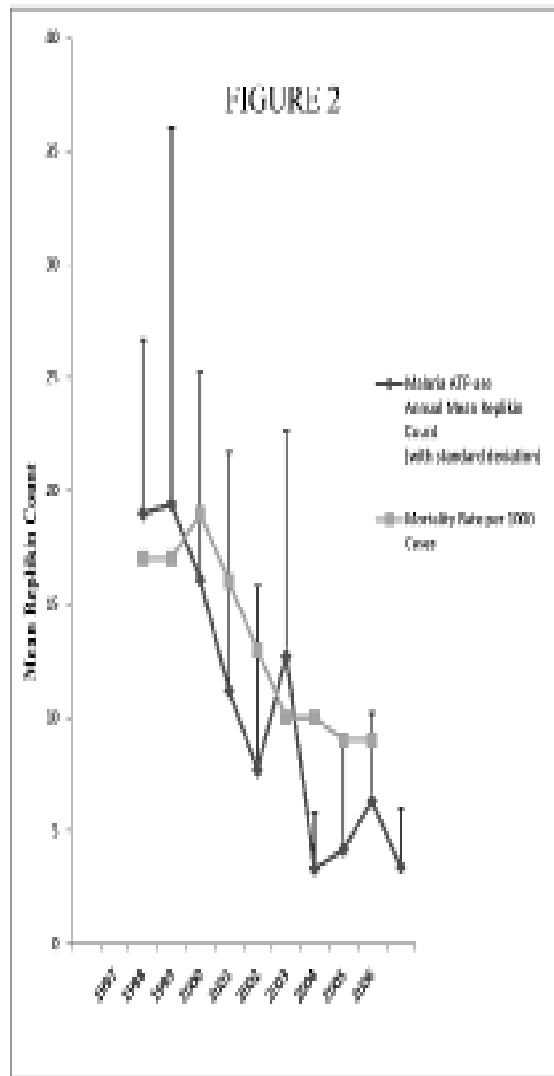
2001					
2002	18	29	>.5	<.3	13
2003	28.4	3	<.001	<.2	7
2004	17	0	<.001		1
2005	7.4	6.5	<.05	<.02	5
2006					
2007	17.2	19	>.5	<.2	8

As is seen in Supplement Figure 1 and Supplement Table 1 above and as also seen in Supplement Figure 2 and Supplement Table 3 below, changes in malaria virulence and mortality may be predicted by identifying a peak within an identified cycle in the Replikin concentration of isolates of a plurality of the trypanosome and predicting an increase in the virulence, morbidity, and/or mortality of a trypanosome of the same species isolated at a time point or time period subsequent to the time point or time period of the identified peak in the cycle of Replikin concentration. In contrast to Figures 3 and 4 for West Nile virus and influenza, morbidity data is not reflected in the analysis of malaria in Figure 1 and is not contained in Figure 2. Use of mortality data and not morbidity data in these Figures and their related analysis and tables is based on the researcher's understanding that morbidity data in malaria is generally unreliable while mortality data is considered more reliable. While the analysis of Supplement Figure 1 and the data in Supplement Figure 2 demonstrate a relationship between Replikin Count in *P. falciparum* and mortality, the skilled artisan will understand that the relationship would also be expected to extend to morbidity and general virulence in malaria just as it has in West Nile virus (see Supplement Figure 3) and influenza.

Cyclic increases in Replikin concentration in the genome can be a mechanism of expansion of an infectious organism into a territory. The Replikin concentration in each Replikin Peak Gene of each Replikin cycle apparently builds on the previous one. In both the mosquito-borne West Nile Virus and mosquito-borne malaria trypanosomes this build-up probably occurs during winter seasons, dry seasons, or otherwise dormant periods. Timely, repeated analyses of cyclic changes in the organism's Replikin structure is useful to bring current the targets for the chemical synthesis of Replikin vaccines having a best fit for emerging pathogens having increased virulence, morbidity, and/or mortality. These strain-specific vaccines may be manufactured in seven days as has been demonstrate with a 91% protection of shrimp against the lethal Taura Syndrome Virus. its entirety by reference).

Malaria trypanosomes were found to have the highest Replikin counts seen to date in any infectious organisms - up to twenty times those in influenza and West Nile Virus. Consistent with these high counts, trypanosomes have one of the highest replication rates in nature. This property may account in part for the resistance of malaria to previous attempts at vaccination. The discovery of the relation of Replikin sequences to rapid

replication offers a new approach, and means, to inhibit rapid replication in



malaria

Figure 2 illustrates that mortality rates per 1000 clinical cases of malaria in humans generally correlate with annual mean Replikin Count in sequences of the *P. falciparum* ATP-ase enzyme publicly available at www.pubmed.com. Mean annual Replikin Counts of *P. falciparum* ATP-ase increased from 1997 to 1998 along with an increase in mortality per malaria case from 1997 and 1998 to 1999. The mean annual Replikin Count of *P. falciparum* ATP-ase decreased from 1998 to 2007 along with the mortality rates from 1999 to 2005 (consistent mortality data is considered presently available only through 2005). Mortality rates per 1000 human cases of malaria for 1997 to 2005 were as follows: 1997 mortality rate was 17; 1998 mortality rate was 17; 1999 mortality rate was 19; 2000 mortality rate was 16; 2001 mortality rate was 13; 2002 mortality rate was 10; 2003 mortality rate was 10; 2004 mortality rate was 9; and 2005 mortality rate was 9. Mortality rates are recorded as declared by the World Health Organization. See www.who.int.

Analysis of Replikin Count in Malaria to Predict Increased Mortality

The authors analyzed publicly available sequences for isolates of *P. falciparum* from PubMed using proprietary search tool software (ReplikinForecast™ available in the United States from REPLIKINS LLC, Boston, MA) from years 1986 to 2007 and determined the mean Replikin Count for the histidine-rich protein of all isolates available in each of those years. The authors then compared the mean Replikin Count for each year with changes in mortality as reported by the World Health Organization

A list of the accession numbers analyzed for the presence and concentration of Replikin sequences is provided in Supplement Table 2 below. The mean Replikin Count for each year is provided following the list of accession numbers from isolates in each corresponding year. Standard deviation and significance as compared to the mean Replikin Count of the previous year and of the lowest mean Replikin Count within the data set are also provided along with the mean Replikin Count for each year

Analysis of Replikin Count in Malaria ATP-ase to Predict Increased Mortality

The authors analyzed publicly available sequences of the ATP-ase enzyme of isolates of *P. falciparum* at www.pubmed.com. The data is summarized below in Supplement Table 3 and illustrated above in Supplement Figure 2. The data illustrate that mortality rates per 1000 clinical cases of malaria in humans correlate with annual mean Replikin Count in sequences of the *P. falciparum* ATP-ase enzyme publicly available at www.pubmed.com. Replikin Counts of The Replikin Count of *P. falciparum* ATP-ase increased from 1997 to 1998 along with an increase in mortality per malaria case from 1997 and 1998 to 1999. The Replikin Count of *P. falciparum* ATP-ase decreased from 1998 to 2007 along with mortality rates from 1999 to 2005 (consistent mortality presently available only through 2005). Mortality rates per 1000 human cases of malaria for 1997 to 2005 were as follows: 1997 mortality rate was 17; 1998 mortality rate was 17; 1999 mortality rate was 19; 2000 mortality rate was 16; 2001 mortality rate was 13; 2002 mortality rate was 10; 2003 mortality rate was 10; 2004 mortality rate was 9; and 2005 mortality rate was 9. Mortality rates are recorded as declared by the World Health Organization. See www.who.int

High malaria morbidity and mortality rates occurred in the late 1990s and were thought to be due to a decreased effectiveness of anti-malarials. ATP-ase is a primary target of artemisinin treatment of malaria. With increased use of artemisinin, and improved public health measures, morbidity and mortality rates declined from 1999 to 2005

Table 3

Year	Mean Replikin Count in	Standard Deviation	Mortality Rate per 1000
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	<i>P. falciparum</i> ATP-ase		Malaria Cases
1997	19	7.7	17
1998	19.4	16.6	17
1999	16.1	9.1	19
2000	11.2	10.5	16
2001	7.7	8.1	13
2002	12.7	9.9	10
2003	3.3	2.5	10
2004	4.2	4.6	9
2005	6.3	3.9	9
2006	3.4	2.6	
2007	6.2	8.4	

Analysis of Replikin Count Cycles in Malaria to Predict Entry into Geographical Regions

The phenomenon of geographical expansion, seen with West Nile Virus entry into North America in 2000 and expansion to the present, also applies to malaria and other pathogens. Analysis of the Replikin concentration of a Replikin Peak Gene, histidine-rich protein, or ATP-ase of *P. falciparum* that demonstrates Replikin concentration cycles may provide a prediction of an expansion of *P. falciparum* mortality and/or morbidity. For example, if a Replikin concentration cycle based on isolates from a particular region of a region demonstrate a prolonged rise in mean annual Replikin Count or a peak following a rise in mean annual Replikin Count, the significant rise or peak predicts an expansion of the mortality rate or morbidity rate of that isolate into contiguous or nearby regions that until the significant rise or peak in Replikin Count did not experience the mortality rate or morbidity rate of the particular region.

A cycle of Replikin concentration is established in the Sahel region of Africa with two peaks at years 2 and 7. The second peak at year 7 is significantly higher than the first peak at year 2 with a p value of 0.01. The Sahel region between years 0 and 7 has experienced a higher rate of mortality than more southerly regions. Based on the higher peak at year 7, it is predicted that the mortality from malaria will increase in the region contiguous to the south of the Sahel. A plurality of Replikin sequences are isolated from year 7 isolates. Replikins that have been conserved between years 0 and 7 are selected as vaccines for malaria in the Sahel and contiguous regions to the south. Replikins that are new in year 7 are likewise selected as vaccines.

Analysis of Replikin Count in Malaria to Predict Increased Mortality

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in each of those years. The authors then compared the mean Replikin Count for each year with changes in mortality as reported by the World Health Organization.

A list of the accession numbers analyzed for the presence and concentration of Replikin sequences is provided in Supplement Table 2 below. The mean Replikin Count for each year is provided following the list of accession numbers from isolates in each corresponding year. Standard deviation and significance as compared to the mean Replikin Count of the previous year and of the lowest mean Replikin Count within the data set are also provided along with the mean Replikin Count for each year.

Analysis of the annual mean Replikin Count of the histidine rich protein between 1986 and 2007 revealed two cycles of Replikin Count. The beginning of the third cycle provides a prediction that Replikin Count may continue to increase along with an increase in malaria mortality rate. The data is graphically illustrated in Supplement Figure 1 and summarized in Supplement Table 1 above.

Analysis of Replikin Count in Malaria ATP-ase to Predict Increased Mortality

The authors analyzed publicly available sequences of the ATP-ase enzyme of isolates of *P. falciparum* at www.pubmed.com. The data is summarized below in Supplement Table 3 and illustrated above in Supplement Figure 2. The data illustrate that mortality rates per 1000 clinical cases of malaria in humans correlate with annual mean Replikin Count in sequences of the *P. falciparum* ATP-ase enzyme publicly available at www.pubmed.com. Replikin Counts of The Replikin Count of *P. falciparum* ATP-ase increased from 1997 to 1998 along with an increase in mortality per malaria case from 1997 and 1998 to 1999. The Replikin Count of *P. falciparum* ATP-ase decreased from 1998 to 2007 along with mortality rates from 1999 to 2005 (consistent mortality presently available only through 2005). Mortality rates per 1000 human cases of malaria for 1997 to 2005 were as follows: 1997 mortality rate was 17; 1998 mortality rate was 17; 1999 mortality rate was 19; 2000 mortality rate was 16; 2001 mortality rate was 13; 2002 mortality rate was 10; 2003 mortality rate was 10; 2004 mortality rate was 9; and 2005 mortality rate was 9. Mortality rates are recorded as declared by the World Health Organization. See www.who.int High malaria morbidity and mortality rates occurred in the late 1990s and were thought to be due to adaptation of the microorganism and decreased effectiveness of anti-malarials. ATP-ase is a primary target of artemisinin treatment of malaria. With increased use of artemisinin, and improved public health measures, morbidity and mortality rates declined from 1999 to 2005.

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