

# **Gene Replikins technology gives evidence of marked mutations of Zika virus in 2016**

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# Replikins

- Gene peptides related to rapid replication
- 7 to 50 amino acids long; sequences, specific amino acids, and spaces between, identified and counted by automated process
- **Replikin concentration**
  - **increases** before **outbreaks**.
  - **decreases** before **cessation**.

# Replikins visualization

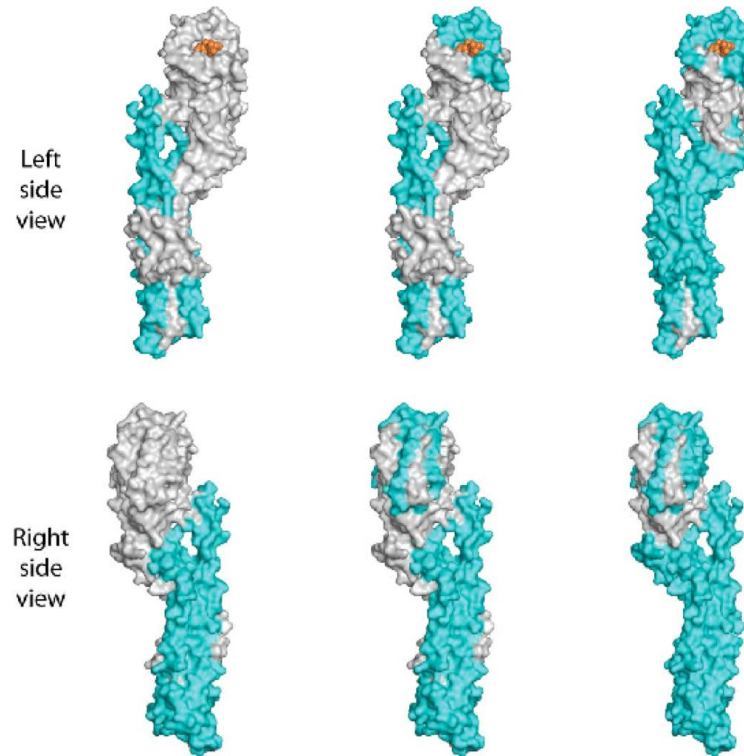
*Replikin Count* = number of Replikins  
per 100 amino acids in gene

Visualized 3 ways:

- 3D x-ray diffraction
- mean and SD of concentration (Replikin Count)
- plotting individual specimen Replikin Counts

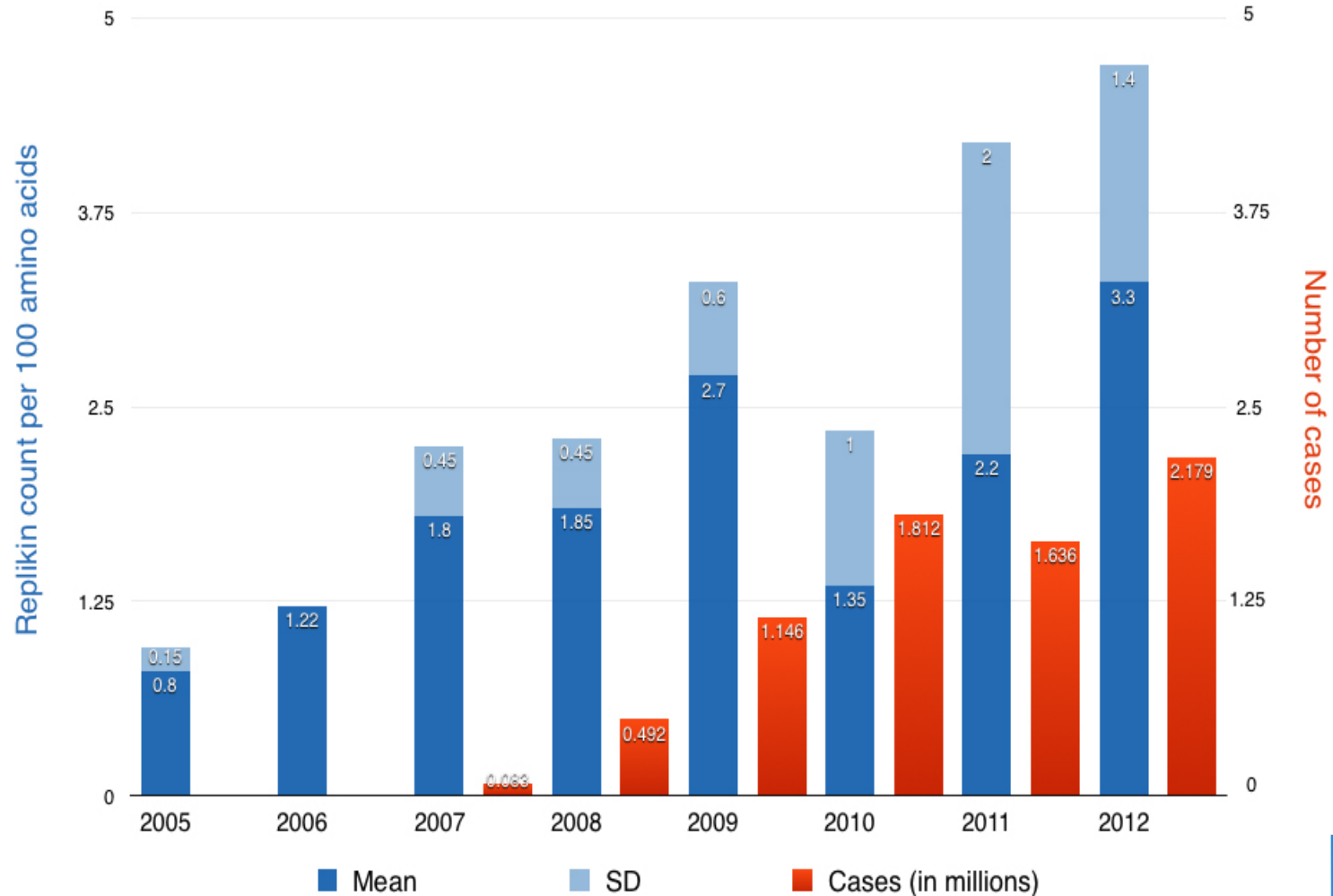
# 3D X-Ray diffraction of H1N1 HA gene increasing before 2009 outbreak

year	2003	2005	2009
Replikin count	3.2	5.5	10.1

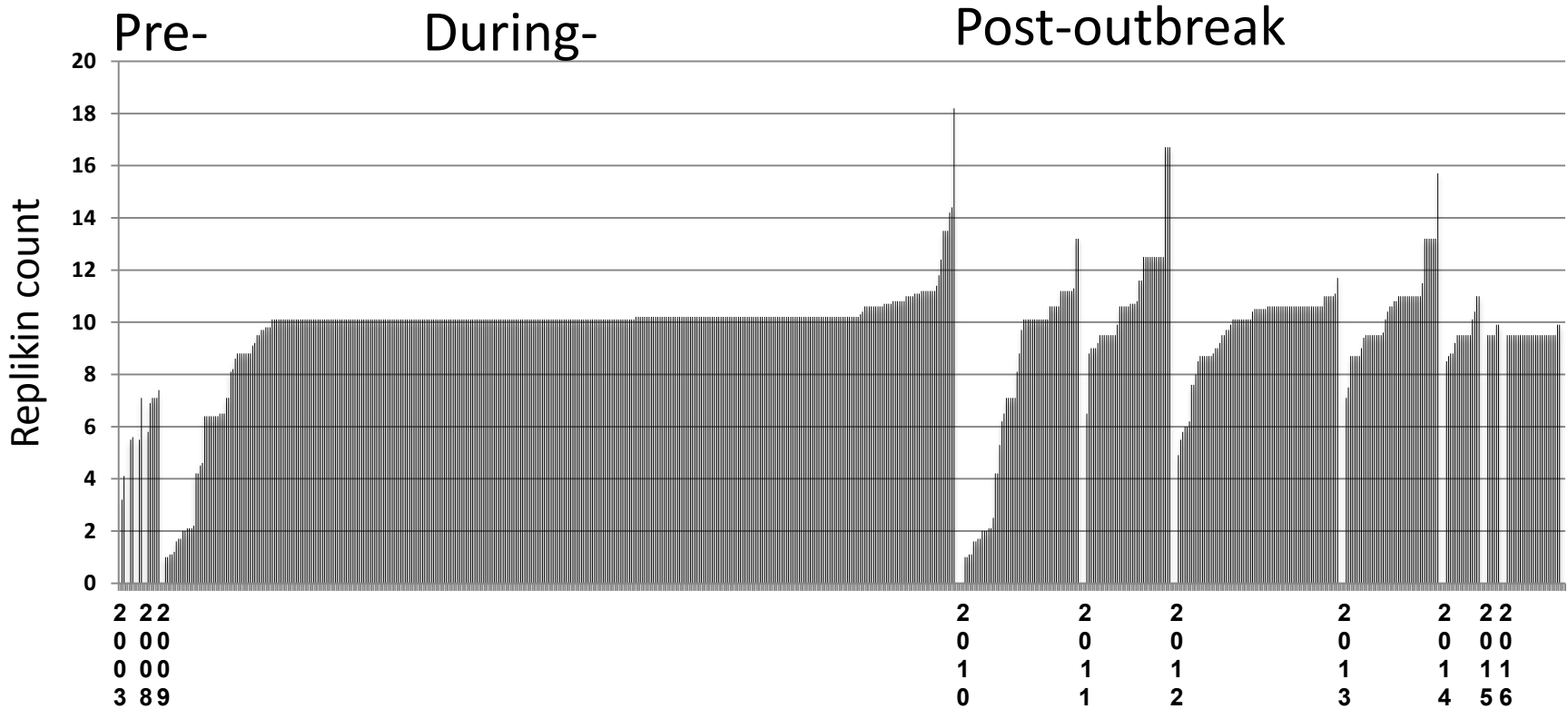


Increasing replikin count correlates with expanding replikin surface area coverage in H1N1 haemagglutinin. Based on sequence alignment between a progression of H1N1 strains with increasing replikin count (3.2, 5.5, 10.1), relative replikin surface area is shown superimposed onto the 1918 H1N1 strain haemagglutinin (grey).

# Mean and SD of Replikin count both increasing before Hand-Foot-and-Mouth disease outbreaks in China



# H1N1 HA Individual specimen Replikin Count plots in Mexico & New Mexico



All individual specimens on Pubmed Pre-, During, and Post H1N1 Pandemic of 2009 (N=635)

# EBOLA individual specimen Replikin count plots in 2013-14 predicted 2014-15 outbreak & cessation

## Ebola Outbreak 2014

All individual specimens each year on Pubmed

Replikin

Count 2013

2014

2015



Number of Specimens (N)

1967-1987 N=20

1990-1999 N=106

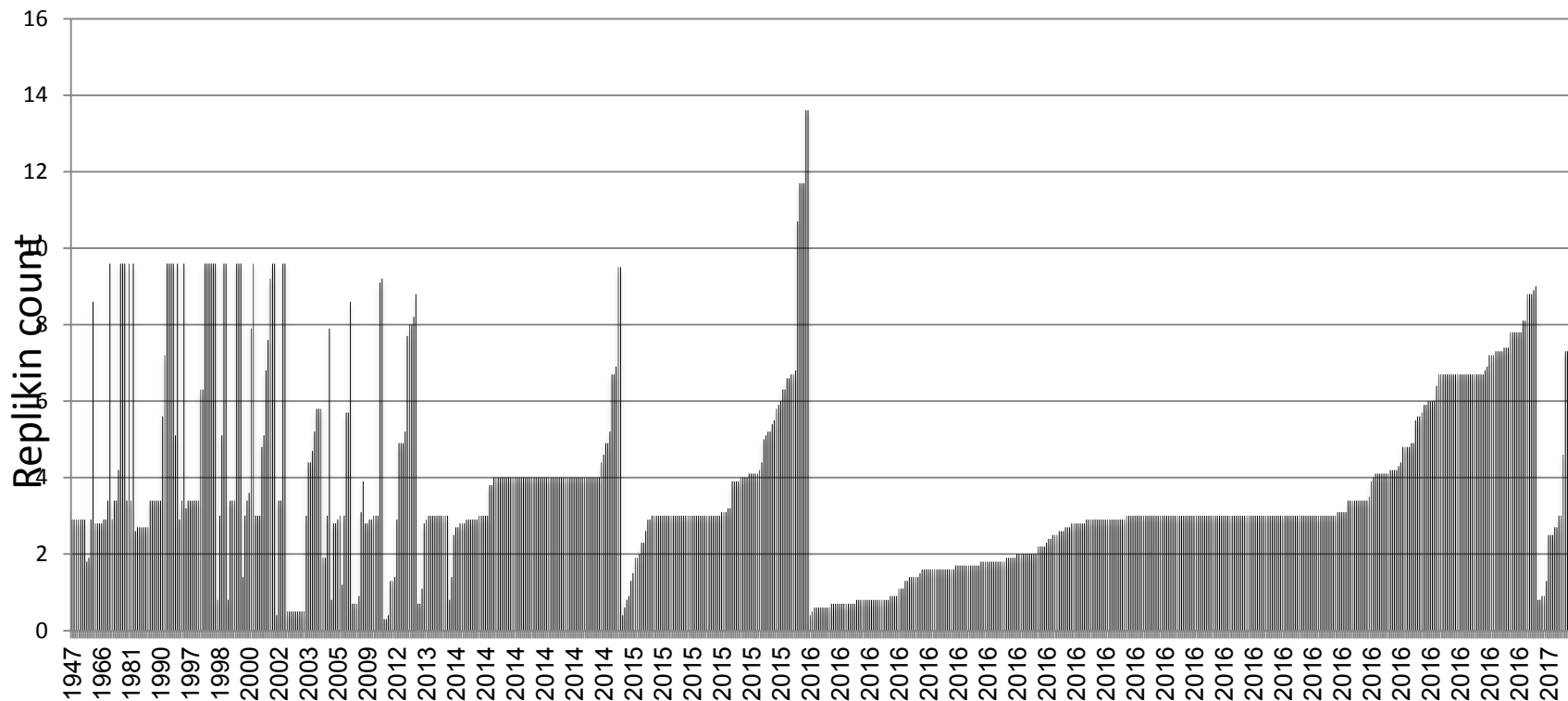
2013 N=22

2014 N= 3107

2015



# ZIKA Individual specimen Replikin Count plots predict outbreaks worldwide



All individual specimens on Pubmed .Feb. 23, 2017 ( N=886)

Increases in Replikin counts precede outbreaks in Africa 1947-2012, French Polynesia 2013, Easter Island 2014, Brazil 2015, Global 2016.

Plateaus in Replikin Counts indicate Replikin Strains.

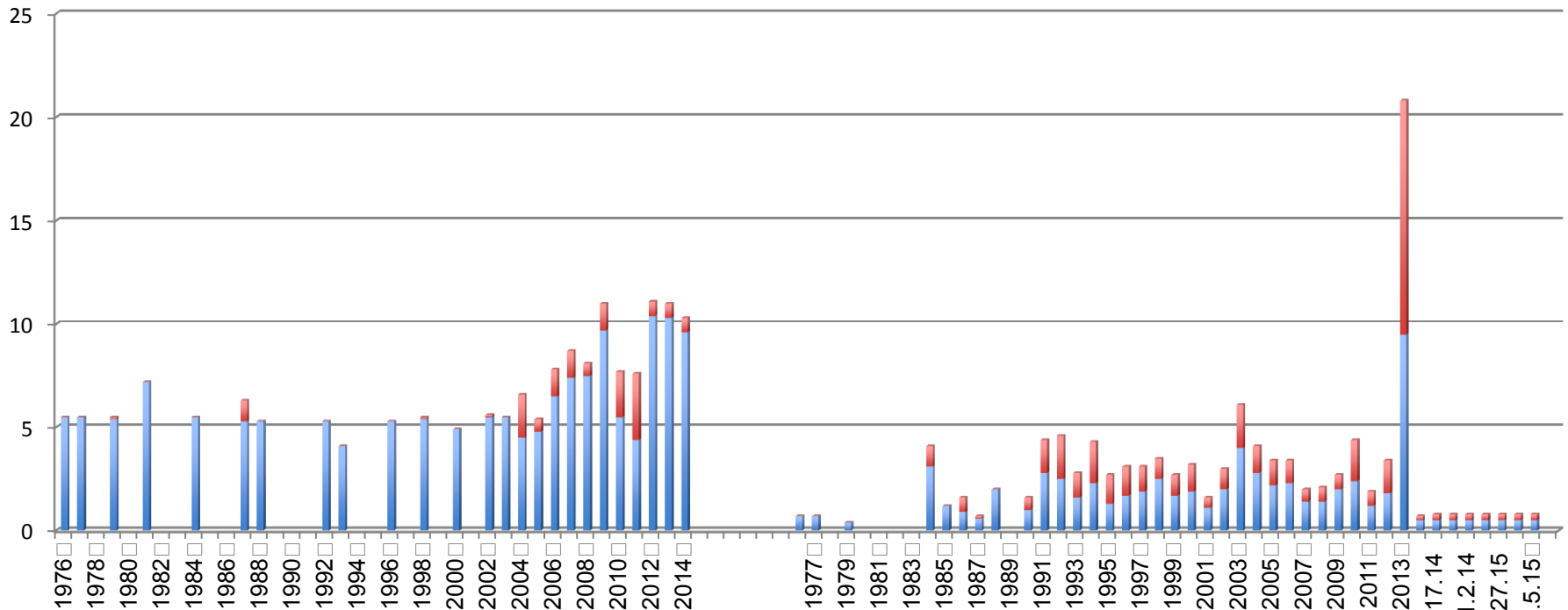




# H1N1 vs. Ebola: Two different courses of onset and progression of outbreak

H1N1 Outbreak in Canada 2009

Ebola Outbreak in Africa 2014



H1N1 (TYPE II Course):

low mortality (~1.3%);

gradual increase in Replikin Count;

followed by gradual small decline;

Clinical recurrence each year (endemic).

Ebola (TYPE I Course):

high mortality (40-70%);

rapid increase in Replikin Count;

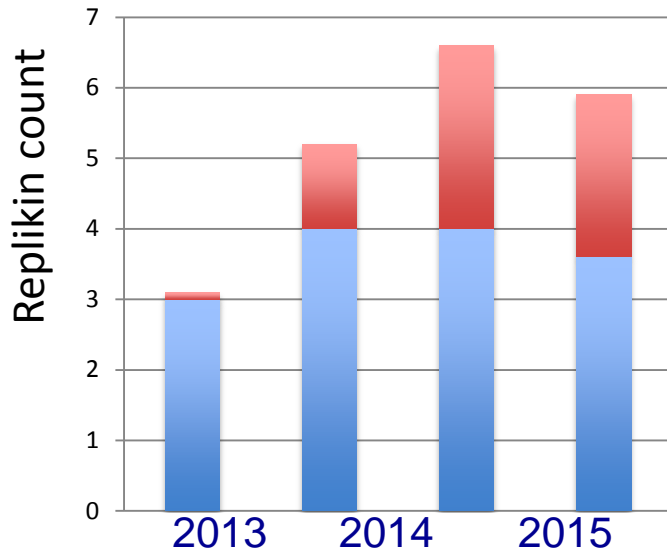
followed by rapid large decline;

No clinical recurrence for two years.



# Zika: Mean & SD increasing since 2013

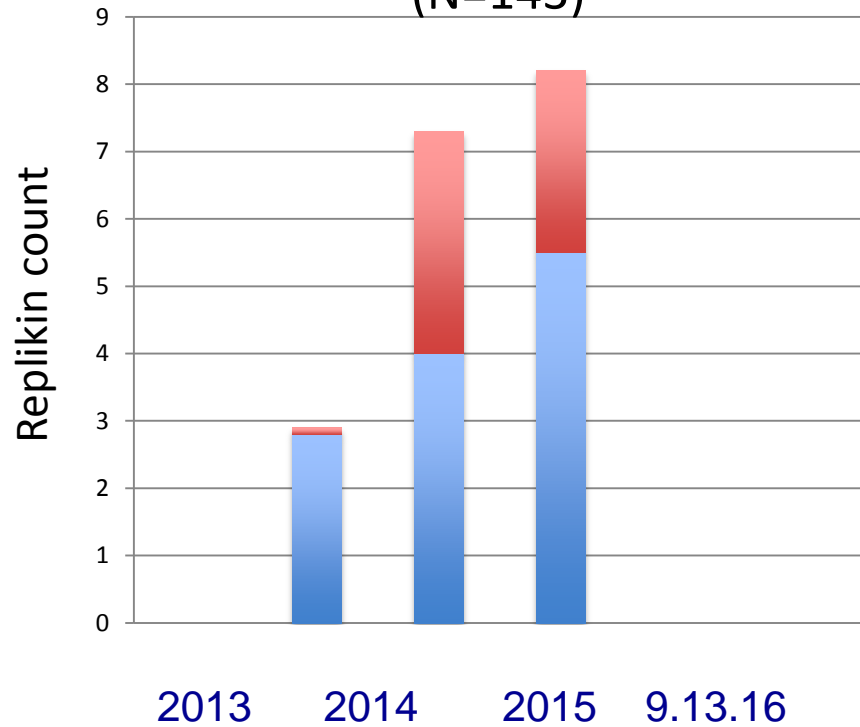
Global  
(N=514)



9.13.16

Significant increase in Replikin Count at  $p < 0.001$ , one year before Zika outbreak.

Brazil  
(N=143)



Mean: blue  
SD: red

2013 2014 2015 9.13.16

Despite slight decline globally, Brazil Replikin Counts continue to increase, indicating possible repeat endemic outbreak in Brazil in coming summer seasons.

All specimens available on PubMed.



- Replikin Technology reduces the pathogen's advantage of complete surprise
- And provides more time for *pre-outbreak* public health responses, and timely “made-to-measure” blockers and vaccines.

## Specific Zika virus gene structures, Replikins, give automated advance warning of outbreaks and their cessation.

Samuel Bogoch and Elenore S. Bogoch

### Abstract

(1st International Conference on Zika, Washington, D.C., Feb. 23, 2017)

Changes in gene Replikin Counts (number of Replikins per 100 amino acids) (1) and compositions provide an automated quantitative method to detect mutations, to measure strain heterogeneity real-time, and to give advance warning of outbreaks and their cessation. Two time-course patterns of gene mutation and molecular evolution observed by gene Replikin Counts, and correlating with clinical course, have been found: **time-course I**: “rapid”, gene mutations (at  $p < 0.001$ ) within 1-2 years in advance of the clinical outbreaks with high mortality, followed by rapid cessation of gene change within the year of the outbreak, followed promptly by rapid cessation of the outbreak, and no recurrence within two years (eg. in Ebola 2014 (2), and SARS 2003); **time-course II** “gradual” gene mutations (at  $p < 0.001$ ) over a period greater than two years in advance of the clinical outbreak, followed by gradual cessation of both the gene change and of the clinical outbreak, followed by annual endemic recurrences (eg. H1N1 influenza pandemic of 2009-10, and Zika 2015). Over 3 million automated direct quantitative measurements have been made to date in 18 virus gene sequences listed annually in Pubmed. and other databases. Changes in Replikin Counts are considered significant at  $p < 0.001$ . The gene Replikins Surveillance method provides advance notice and time to respond to outbreaks before they occur, beginning to remove from the emerging lethal organism the advantage of complete surprise with regard to when, where, and with what gene sequences it may next rapidly replicate in a particular host.

References: 1. Nature Precedings doi:10.1038/npre.2011.6279.1;doi: 10.1038/npre .2012.7144.1. 2. Marked Increase in Ebola Gene Replikin Count in 2012 & 2013 Predicted Current Ebola Outbreak; Does Recent Sharp Drop in Replikin Count Signal Early End for the Current Ebola Outbreak? Reuters, October 4, 2014.



# ABSTRACT UPDATE Feb.20.2017

## Zika Gene Mutations Are Increasing 2016-2017

Zika gene mutations increased markedly in 2016 as evidenced by the appearance of new Zika Replikin Strains.

By different methods, before 2016, only 3 Zika strains in 2000-2010, and 12 strains in 2010-2015 (14/15 'new') were detected (Maron, D.F. Scientific American, May, 2016) .

As of December 4, 2016, for the year 2016, in our findings, when the number of specimens reported on Pubmed was 611, there were 49 Zika Replikin Strains.

As of February 20, 2017, when the total number of specimens reported on Pubmed was 882, there were 54 Zika Replikin Strains.

If the apparently high Zika gene mutation rate continues, an increasingly new group of Replikin Strains may appear in 2017, and the risk will increase that vaccines based on Zika virus gene strains detected prior to 2016, when released in the future, may be less effective.

“There is special providence in the fall of a sparrow.  
If it be now, ‘tis not to come;  
if it be not to come, it will be now;  
if it be not now, yet it will come -----

The readiness is all.”

Hamlet Act 5, scene 2, 217-224

# Contact for Questions

## Replikins Gene Surveillance Service™



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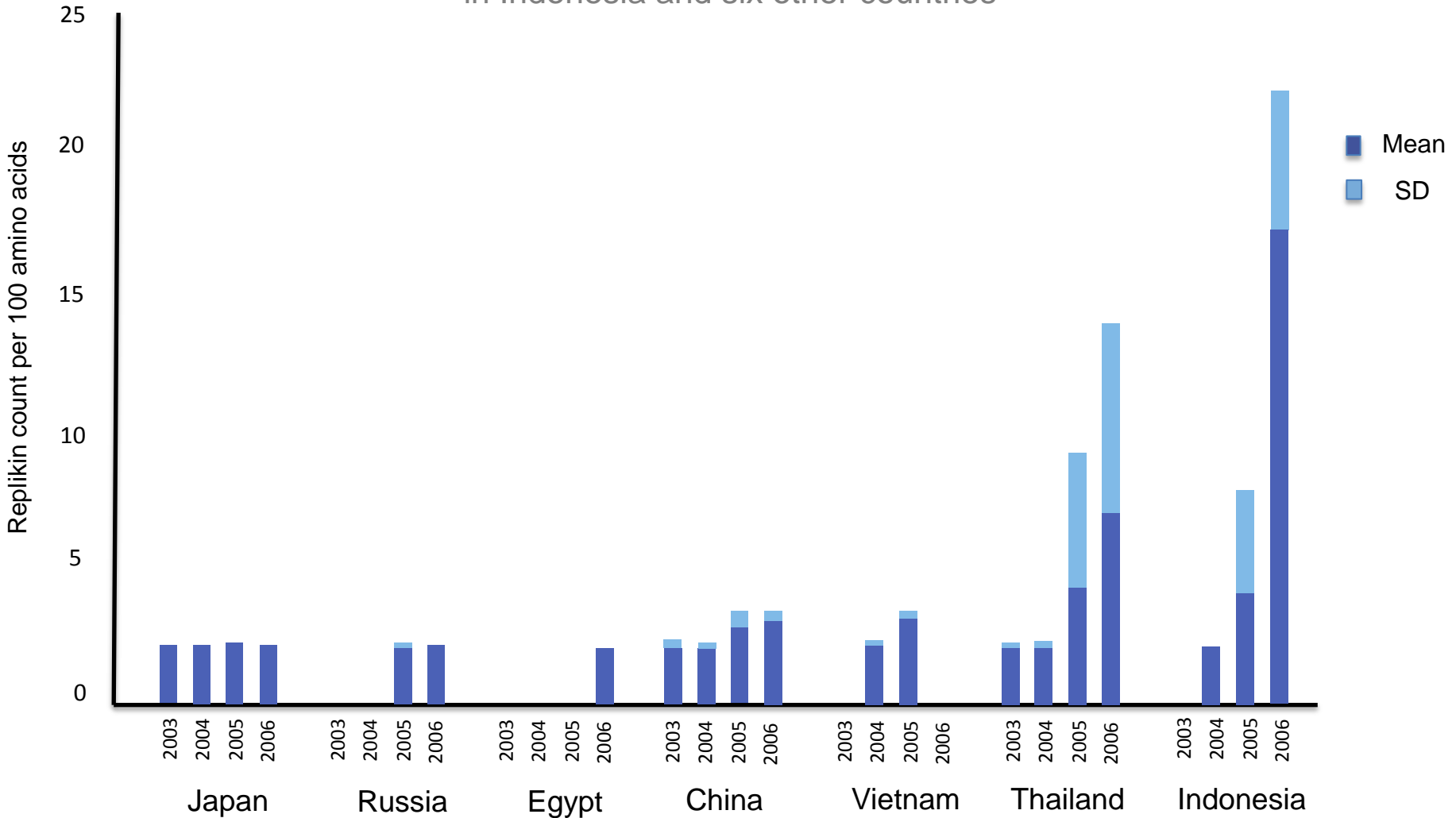
Additional Slides Available



# GEOGRAPHIC LOCALIZATION

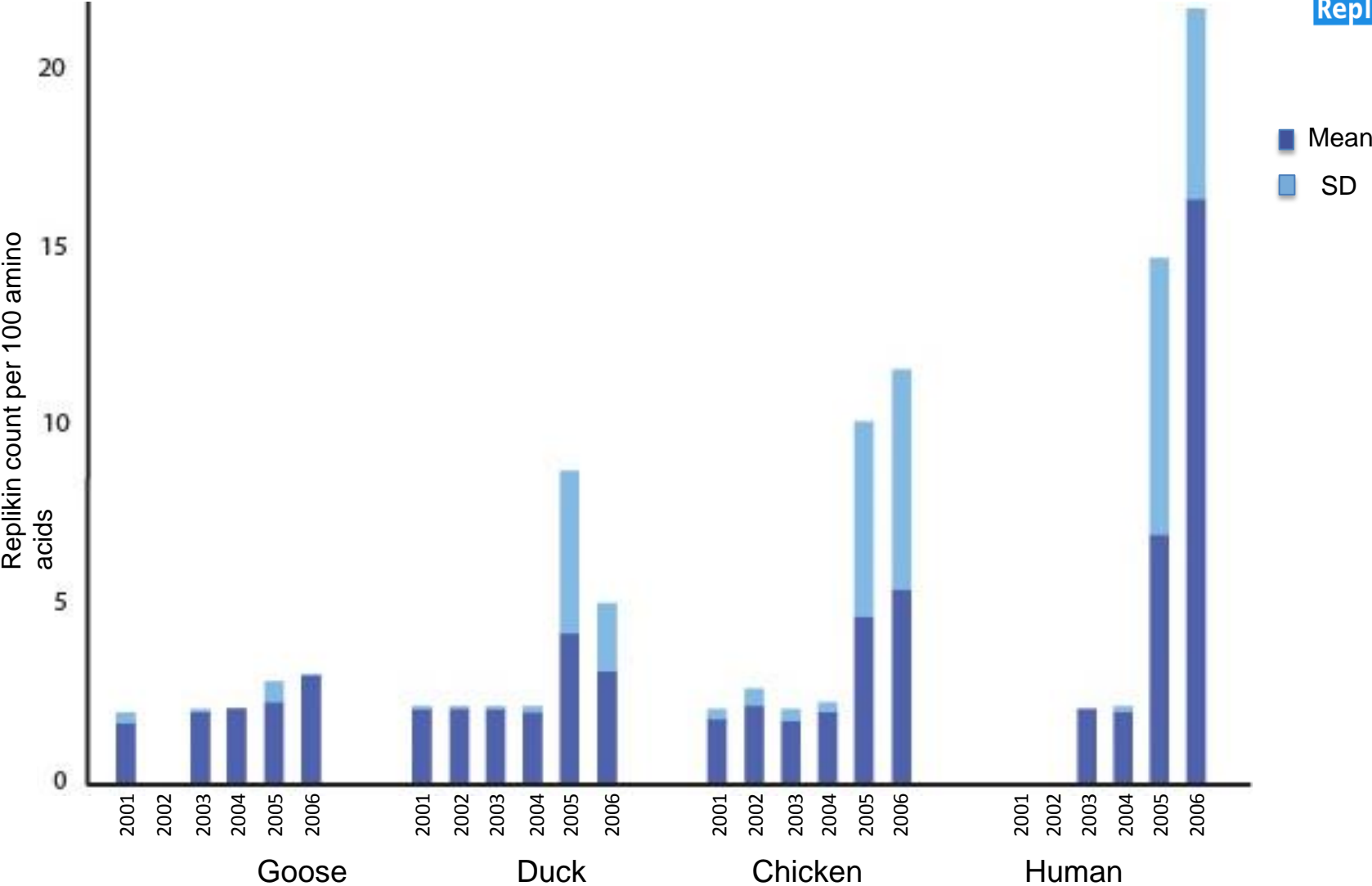


Localization of Replikin Peak Gene of Human H5N1 Virus  
in Indonesia and six other countries



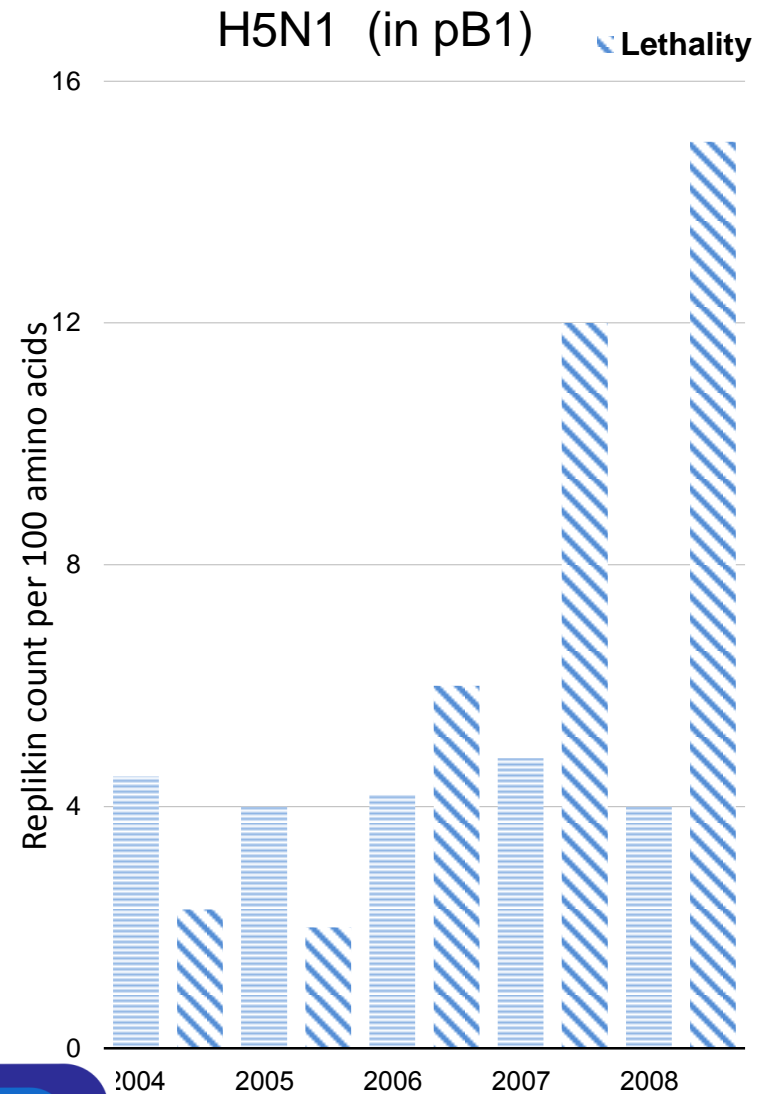
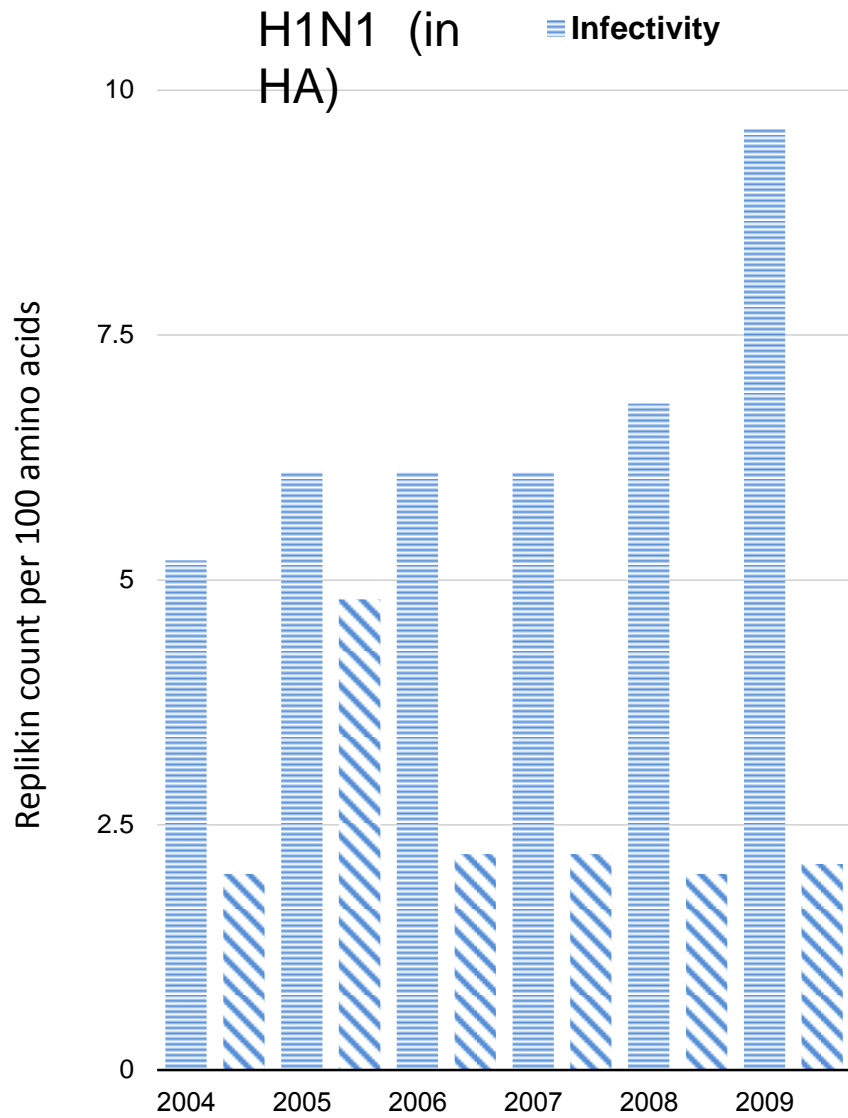
Increase in Replikin Counts in H5N1 in neighboring countries 2003-2006 predict and correlate with outbreaks in those countries. Mortality rate in Indonesia in 2006 reached 82%.

# HOST LOCALIZATION



Replikin Peak Gene of H5N1 Virus activity in four hosts

# Double differentiation: Replikin Counts of infectivity and lethality Genes in H1N1 and H5N1

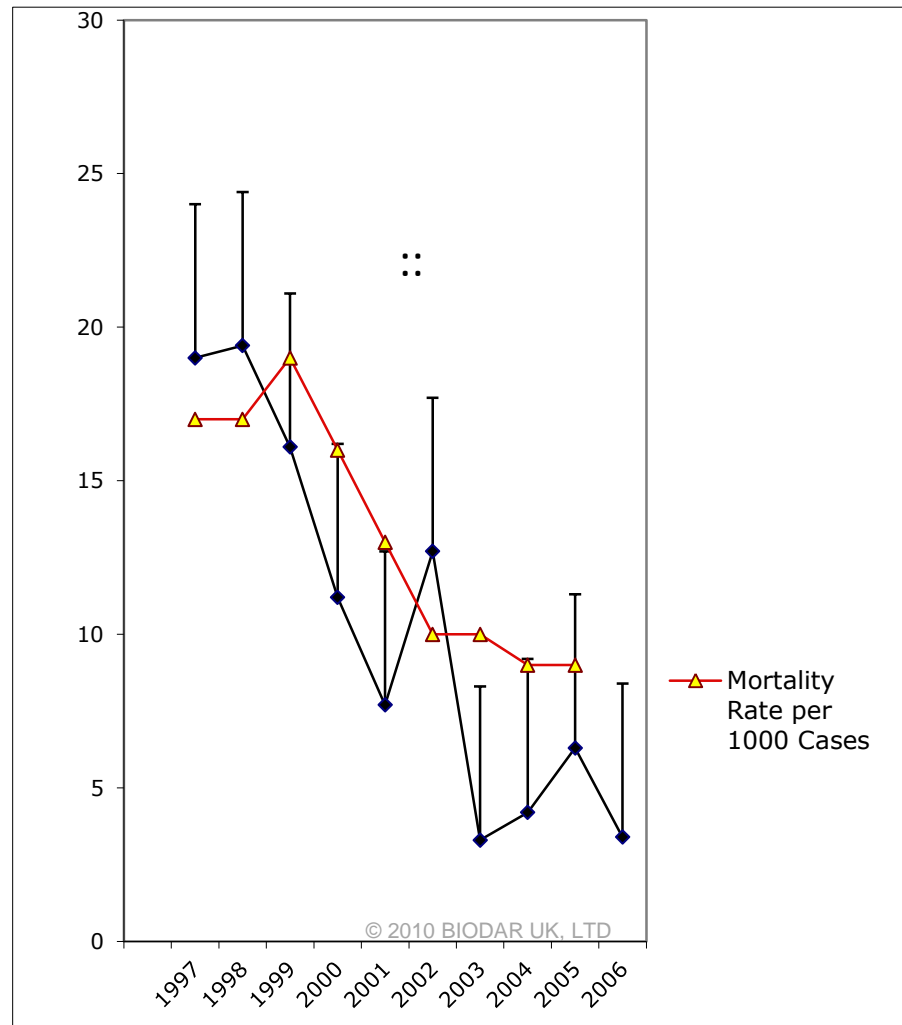


# CORRELATION BETWEEN REPLIKIN DATA AND EPIDEMIOLOGICAL LETHALITY DATA



## MALARIA

Annual Replikin Count (**black**) correlates with annual human mortality rate (**red**, per WHO) in Malaria (*Pl. falciparum*)

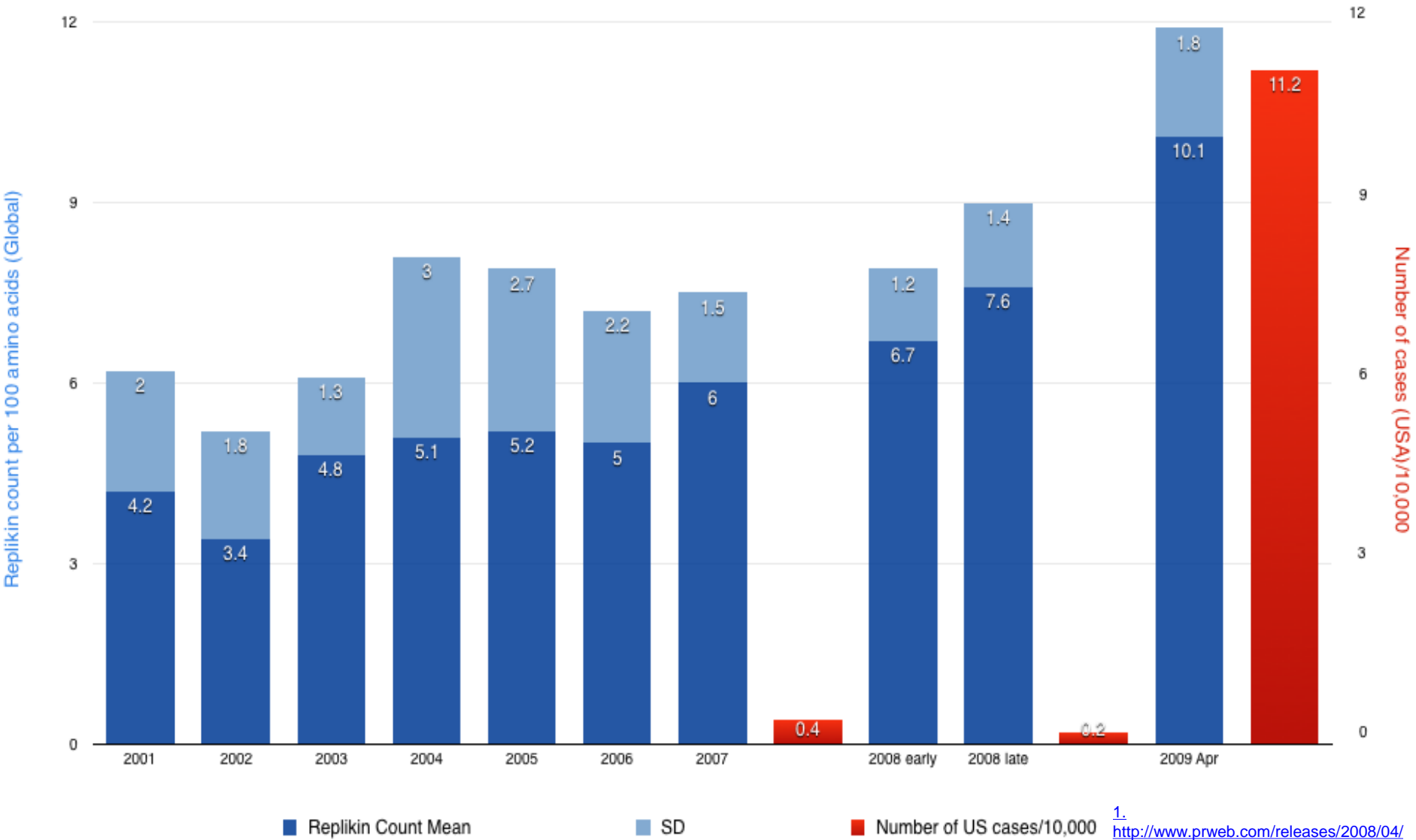


# REPLIKIN COUNT INCREASE PREDICTS MEXICO- U.S. OUTBREAK AND H1N1 PANDEMIC (2001-2009)



“April 2008, Replikin warns:  
 Replikin count “Increase to 7.6 (plus/minus 1.4) highest level since the 1918 H1N1 pandemic...”<sup>1</sup>

June 11, 2009 WHO declares H1N1 Pandemic<sup>2</sup>



1. <http://www.prweb.com/releases/2008/04/>  
 2. <http://www.cdc.gov/h1n1flu/who/>

# REPLIKINS PREDICTS 2009 H1N1 PANDEMIC OVER A YEAR IN ADVANCE



Replikins publishes warning on April 7, 2008 of H1N1 Pandemic threat:

“H1N1 Influenza Virus With Highest Replikin Count™ Since the 1918 Pandemic Identified in the U.S. and Austria ([April 7, \(2008](#)

Replikins, Ltd. has found that the Replikin Count™ of the H1N1 strain of influenza virus has recently increased to 7.6 (plus/minus 1.4), its highest level since the 1918 H1N1 pandemic (p value less than 0.001). A rising Replikin Count of a particular influenza strain, indicating rapid replication of the virus, is an early warning which has been followed consistently by an outbreak of the specific strain...”

<http://www.prweb.com/releases/2008/04/prweb835974.htm>

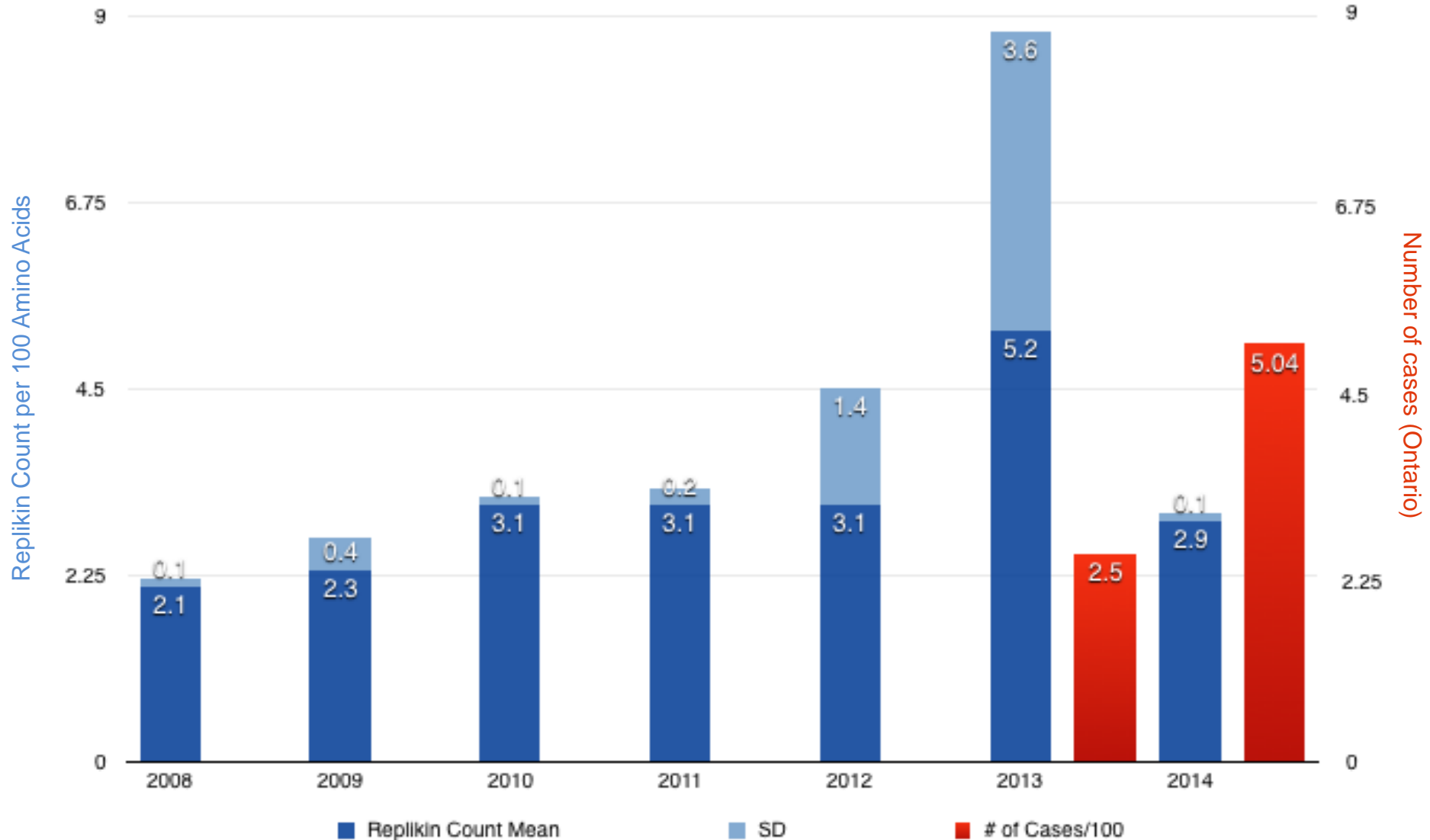
[WHO declares H1N1 Pandemic 14 months later: June 2009 ,11](#)

“On [June 11, 2009](#), the World Health Organization signaled that a global pandemic of novel influenza A (H1N1) was underway by raising the worldwide pandemic alert level to PHASE 6.”

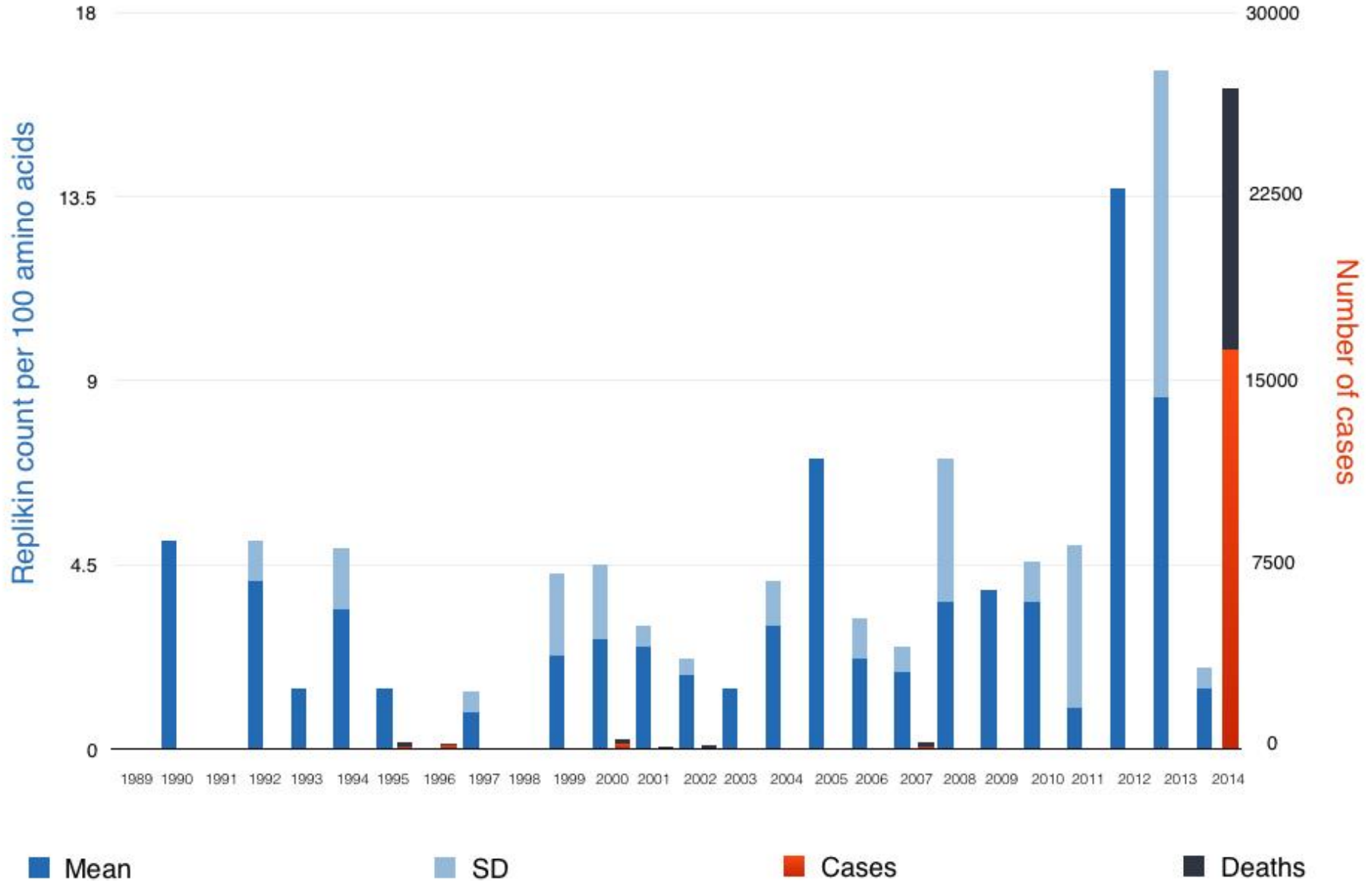
<http://www.cdc.gov/h1n1flu/who/>

[http://www.who.int/mediacentre/news/statements/2009/h1n1\\_pandemic\\_phase6\\_200906](http://www.who.int/mediacentre/news/statements/2009/h1n1_pandemic_phase6_200906)

# REPLIKIN COUNT INCREASE PREDICTS H3N2 OUTBREAK IN ONTARIO CANADA



# REPLIKIN COUNT INCREASE PREDICTS 2014 EBOLA OUTBREAK







# REPLIKINS and CDC's EBOLA PREDICTIONS

September 26, 2014: The CDC Predicts 1,400,000 cases of Ebola could occur by January 2015:

“The ebola epidemic could claim hundreds of thousands of lives and infect more than 1,400,000 people by the end of January, according to a statistical forecast released this week by the U.S. Centers for Disease Control and Prevention. [CDC]” Science Daily, September 26, 2014

October 9, 2014 Replikins publishes that sharp drop in Replikin Count in Ebola gene signals early end to the Ebola outbreak.

LONDON, U.K., Oct. 9, 2014 (SEND2PRESS NEWSWIRE) — Replikins Global Surveillance System™ analysis of the Ebola virus show the highest ever peaks in Ebola Gene Replikin Counts in the years 2012 and 2013. Such genomic increases predicted the current worst ever Ebola outbreak. **A recent sudden drop in the Replikin Counts may signal an early end to the current outbreak** if adequate public health measures are implemented for containment of Ebola virus already in human hosts. <http://finance.yahoo.com/news/marked-increase-ebola-gene-replikin-212900060.html>

Total number of cases as of January 25, 2015 per WHO : 22,092

<http://apps.who.int/ebola/en/ebola-situation-report/situation-reports/ebola-situation-report->