Improving global influenza surveillance: trends of A(H5N1) virus in Africa and Asia

Magdalena Escorcia¹, Matias S Attene-Ramos², Marco Juarez Estrada¹ and Gerardo M Nava³*

Abstract

Background: Highly pathogenic avian influenza A(H5N1) viruses are an important health problem in many Asian and African countries. The current increase in human cases demonstrates that influenza A(H5N1) is still a significant global pandemic threat. Many health organizations have recognized the need for new strategies to improve influenza global surveillance. Specifically, the World Health Organization through the global technical consultation for influenza surveillance have called for a detailed picture of the current limitations, especially at the nation level, to evaluate, standardize and strengthen reporting systems. The main goal of our study is to demonstrate the value of genetic surveillance as part of a strategic surveillance plan. As a proof of concept, we evaluated the current situation of influenza A(H5N1) in Asian and Africa.

Results: Our analysis revealed a power-law distribution in the number of sequences of A(H5N1) viruses analyzed and/or reported to influenza surveillance networks. The majority of the Asian and African countries at great risk of A(H5N1) infections have very few (approximately three orders of magnitude) sequenced A(H5N1) viruses (e.g. hemagglutinin genes). This suggests that countries under pandemic alert for avian influenza A(H5N1) have very limited participation (e.g. data generation, genetic analysis and data share) in avian influenza A(H5N1) surveillance. More important, this study demonstrates the usefulness of influenza genetic surveillance to detect emerging pandemic threat viruses.

Conclusions: Our study reveals that some countries suffering from human cases of avian influenza have limited participation (e.g. genetic surveillance or data share) with global surveillance networks. Also, we demonstrate that the implementation of genetic surveillance programs could increase and strengthen worldwide epidemic and pandemic preparedness. We hope that this work promotes new discussions between policy makers and health surveillance organizations to improve current methodologies and regulations.

Findings

The recent outbreaks of highly pathogenic avian influenza A(H5N1) virus in numerous countries in Asia and Africa and the increase in human cases, demonstrate that influenza A viruses remain a global pandemic threat [1,2]. Worldwide, natural migrations of birds and commercialization of poultry product are considered two of the most important mechanisms of disease dispersion [3]. Due to the high risk of the A(H5N1) pandemic threat, multinational efforts have been made to improve the surveillance and control of avian influenza viruses around the world [4]. In recent years, important progress has been accomplished in the standardization of laboratory techniques for the diagnostics of influenza viruses [5], and phylogenetic nomenclature of highly pathogenic avian influenza A(H5N1) virus [6]. These efforts have generated establishment of advanced global networks for influenza surveillance [7].

In response to the 2009 influenza pandemic threat, the World Health Organization (WHO) through the global technical consultation for influenza surveillance has called for a detailed picture of current limitations in national reporting systems [8,9]. An important component of the influenza surveillance system that deserves significant attention is the monitoring of genetic and antigenic changes occurring in influenza viruses that circulate among human and animals (e.g. [10,11]).
Genetic surveillance of influenza viruses have shown to be an essential tool for establishing the gene origin of pandemic viruses, monitoring the status of an outbreak [11,12] and design control strategies [13,14]. However, the regular use of genetic influenza surveillance remains limited to only a few countries. Herein, we evaluate the extent of this problem and demonstrate the importance of genetic surveillance during the current situation of highly pathogenic avian influenza A(H5N1) virus in Asia and Africa. Given the significance of this global pandemic threat, this study provides some basis to improve our current surveillance programs.

**Surveillance of avian influenza H5N1 virus in Asia and Africa**

Information of human cases of avian influenza A(H5N1) reported to the WHO, were retrieved from the Global Alert and Response (GAR) system [15]. This information has been collected under WHO policies and regulations, and includes epidemiological data and operational information about disease outbreaks. This system manages critical information about outbreaks and accurate and timely communications between international public health professionals [16]. A written consent (WHP-73,056) to extract and publish the data (number human cases) was obtained from the WHO.

Avian influenza A(H5N1) have been reported in 15 countries (as of 25th March, 2011): Azerbaijan, Bangladesh, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Lao People’s Democratic Republic, Myanmar, Nigeria, Pakistan, Thailand, Turkey and Viet Nam [15]. Because other countries in Asia, Africa and Europe are at prominent risk of influenza outbreaks, the global network for influenza surveillance has implemented specific surveillance polices for this region [7]. To gain insights into the current status and capacity of influenza A(H5N1) surveillance in these countries, we analyzed the number of A(H5N1) hemagglutinin (HA) sequences generated in national disease centers and universities as an indicator of active surveillance. Analysis of influenza HA proteins is one of the most important tools for global influenza surveillance network [2,17].

First, we examined the number of deposited HA sequences in the Influenza Virus Resource, a comprehensive database integrating data from the National Institute of Allergy and Infectious Diseases (NIAID), the J. Craig Venter Institute (JCVI) and GenBank at the National Center for Biotechnology Information (NCBI) [18]. We retrieved and archived records for HA sequences in Asian and African countries (all sequences available from different hosts and environmental samples) of influenza A virus subtype H5N1 from 2003 to 2011 (as of 25th March, 2011). A total of 2,934 HA sequences were found and retrieved from the database. We found that the cumulative number of sequences for this period of time (2003-2011) varied drastically between countries (Figure 1). The analysis revealed an approximation to a power law distribution showing some degree of inequality between nations. For example, most of the A(H5N1) sequences (73.7%) were obtained from a few number of countries (China, Egypt, Vietnam, Thailand and Indonesia) and of the 36 Asian and African countries that have performed genetic surveillance of HA proteins, 50% of them have deposited less than 10 sequences in the database between 2003 and 2011 (Additional file 1).

To verify the power law approximation in influenza genetic surveillance, we performed two independent statistical tests. First, we ranked the countries by the number of available sequences and then we fit the data to a nonlinear power law regression and a log-log plot using GraphPad Prism 4 software, (GraphPad Prism, CA. USA). Second, we performed the Kolmogorov-Smirnov test for goodness-of-fit to power law distributions [19] derived from data variability using the lower (25th percentile), middle (50th percentile or median), and upper (75th percentile) quartiles. This statistical analysis was carried out using StatView software version 5.0.1. (SAS Institute, NC. USA). These analyses confirmed that HA gene sequences archived in the Influenza Virus Resource have an approximation to a power law distribution (nonlinear power law regression, \( R^2 = 0.9066 \) and Kolmogorov-Smirnov test \( P < 0.0001 \) for lower, middle, and upper quartiles; Figure 2) demonstrating a strong sequencing disproportion between nations. Together, these data suggest that some of the selected nations have limited participation (e.g. data generation, genetic analysis and data share) in the genetic surveillance of avian influenza A(H5N1) or a limited interaction with global surveillance networks for data share. These observations highlight the need of new
policies to improve collaboration between nations affected by influenza outbreaks.

Second, to evaluate the trends of influenza genetic surveillance in countries under current avian influenza A (H5N1) outbreaks in humans, we examined the number of reported HA sequences from the 15 countries with avian influenza A(H5N1) disease cases and deaths (as of 25th March, 2011) [15]. Our analysis demonstrated that between the years 2003 and 2011, nations such as China, Egypt, Indonesia, Thailand and Viet Nam have performed a constant A(H5N1) genetic surveillance (Figure 3). Intriguingly, we observed instances of lack of A(H5N1) genetic surveillance even when human cases and deaths took place (e.g. Indonesia 2008, 2010, 2011 and Egypt 2011; Viet Nam 2009, Cambodia 2009, 2010, 2011). These results indicated that in many instances, genetic analysis of influenza A(H5N1) is used as an Ad hoc strategy rather than a surveillance method. Moreover, our results also demonstrate that genetic surveillance of influenza viruses is performed by disease control centers and research institutions with a limited number of sequences per year (e.g. Cambodia, 37 HA sequences in 8 year period).

To demonstrate that some countries suffering from human cases of avian influenza have limited participation (i.e. genetic surveillance or data share) with global surveillance networks, we estimated the ratios between total number of avian influenza A(H5N1) HA sequences and total number of human cases of influenza A(H5N1) (data collected between 2003 and 2011, as shown in Figure 3). This analysis revealed significant differences ($P \leq 0.016$) in sequencing capability between countries (Figure 4). For example, in Nigeria 171 A(H5N2) genes were sequenced per human case; in contrast, in Azerbaijan only 0.6 genes were sequenced per human case. These numbers represent a 285 fold difference between these two countries. Together, these findings also indicate that some nations have limited participation in the
human influenza outbreaks and their involvement with the current situation of some nations facing the risk of surveillance may occur in some of these countries. Deposition in influenza global networks, and that serological 2011) A(H5N1) viral isolates have close homology to Figure 5, cluster A). In many countries, recent (2008-2007, and in Bangladesh between 2007 and 2010 (e.g. example, viruses circulating in China between 2000 and 2011). A(H5N1) viruses clustered by country and year of isolation selected Asian and African countries, revealed that A(H5N1) viruses circulating between 2000 and 2011 in the region represents an important source of highly pathogenicity and exotic H5N1 viruses isolated from poultry seized at a port of entry into Viet Nam [28]. Genotype III (e.g. China 2006, ADG59089), an exotic virus generated through multiple reassortment events isolated in China. Importantly, viruses with close HA protein homology to genotype III, reacted poorly with antisera produced from other viruses circulating in China. Based on these observations, these viruses were cataloged as a pandemic threat [29]. Genotype IV (e.g. Egypt 2008, ADG28679; and 2010, ADM85867, ADM85879) includes recent isolates of highly pathogenicity avian influenza A (H5N1) viruses circulating in Egypt. Genotype V (USA 2007, ACZ36900 and ACZ48494) represents A(H5N1) sequences used to validate the efficacy of this genetic analysis to detect exotic genotypes. Genotype VI (e.g. Viet Nam 2008, ACY06607, ACY06606, ACO07033 and ADF83652) integrated by high pathogenicity A(H5N1) viruses isolated from shell washes of duck and goose eggs confiscated from travelers. These viruses have close homology to a Hong Kong 97-like H5N1 influenza virus [27]. Genotype II (e.g. Viet Nam isolates from 2008 to 2011, ACO07034, ACO07035, ACO07036 and ACO07037) includes highly pathogenicity and exotic H5N1 viruses isolated from poultry seized at a port of entry into Viet Nam [28]. Genotype II (e.g. Viet Nam isolates from 2008 to 2011, ACO07034, ACO07035, ACO07036 and ACO07037) includes highly pathogenicity and exotic H5N1 viruses isolated from poultry seized at a port of entry into Viet Nam [28]. Genotype III (e.g. China 2006, ADG59089), an exotic virus generated through multiple reassortment events isolated in China. Importantly, viruses with close HA protein homology to genotype III, reacted poorly with antisera produced from other viruses circulating in China. Based on these observations, these viruses were cataloged as a pandemic threat [29]. Genotype IV (e.g. Egypt 2008, ADG28679; and 2010, ADM85867, ADM85879) includes recent isolates of highly pathogenicity avian influenza A (H5N1) viruses circulating in Egypt. Genotype V (USA 2007, ACZ36900 and ACZ48494) represents A(H5N1) sequences used to validate the efficacy of this genetic analysis to detect exotic genotypes. Genotype VI (e.g. Viet Nam 2008, ACY06607, ACY06606, ACO07033 and ADF83652) integrated by high pathogenicity A(H5N1) viruses isolated from chickens seized at ports of entry in Viet Nam [30]. These viruses were antigenically distinct from contemporary strains circulating in Viet Nam. This genotypic group also included A(H5N1) viruses isolated from humans possessing unknown virulence determinants involved in high pathogenicity [31]. Together, these analyses reveal the value of genetic surveillance to identify potential influenza pandemic threats circulating in specific global regions.

The influenza pandemic in 2009 highlighted the need of an improved global surveillance system. Specifically, the last pandemic event showed the technical challenges that many nations face to monitor influenza outbreaks when standardized surveillance and reporting system are not established [8]. Significant efforts have been taken by independent research groups and the WHO to revise and standardize methodologies for data collection and reporting systems. However, these challenges are very complex in
nature and many of them remain ambiguous. For example, the WHO global technical consultation for influenza surveillance and other WHO influenza program experts recently indentify that the lack of standardization in surveillance methods results in data sets that are not comparable between nations and that the data cannot be compiled to generate a comprehensive picture of the virus spread between nations [8]. To this end, our study clearly documents this issue. Here, we have shown that many countries where highly pathogenic avian influenza A (H5N1) virus represents a current pandemic threat, have a limited participation (e.g. data generation, genetic analysis and data share) in avian influenza A(H5N1) surveillance. Although we cannot provide conclusive evidence explaining these national trends, we presume that this issue could be linked to the fact that some nations lack technical sources to perform the surveillance, lack an effective mechanism for reporting surveillance data, have limited human sources (trained personnel, problems with internet connectivity, etc.) or are influenced by political pressures [8].

Importantly, the WHO global technical consultation for influenza surveillance has also called for the description of a detailed picture of the current limitations, especially at the nation level [8,9]. With this in mind, here we provide some bases to establish a strategy to overcome some of these limitations and enhance influenza surveillance. Specifically, our analysis demonstrates that implementation of genetic surveillance programs could strengthen worldwide epidemic and pandemic preparedness. For example, our study revealed that A(H5N1) viruses clustered by country and year of isolation indicating that this virus follows regional and annual trends. Also, our analysis identified highly diverse (exotic) viruses co-circulating with endemic viruses. Based on these results, we propose that genetic surveillance systems should be a key component of influenza surveillance. In particular, we believe that genetic surveillance can be adopted worldwide even in less-resourced areas. This idea is supported by studies demonstrating that a few genetic sequences (less than 50 HA genes sequenced) are sufficient to effectively track the antigenic changes occurring in influenza viruses.

**Figure 5** The genetic diversity and distinctiveness of influenza A(H5N1) viruses circulating between 2003 and 2011 in Asian and African countries. Filled circles depict viruses isolated in 2010-2011.
circulating in specific geographical locations and over a period of 5-10 years [13,25]. We hope the present study stimulates further studies and discussions to accelerate the improvement of guidance and recommendations for influenza surveillance.  
In summary, the significance of the present study is at least three fold. First, it is revealed that in many countries under current pandemic alert for avian influenza A (H5N1), there is a limited genetic surveillance of influenza A(H5N1) viruses. This is due apparently to a limited participation (e.g. data generation, genetic analysis and data share) in avian influenza A(H5N1) surveillance. Second, analysis of national surveillance trends revealed that genetic analysis of influenza viruses occurred in many cases as Ad hoc strategy but not as a surveillance technique (prevention). Third, it is demonstrated the usefulness of influenza genetic surveillance to detect pandemic threat viruses. The significance of this molecular approach was demonstrated in the past A(H1N1) 2009 world pandemic, in which genetic analyses were essential to establish the origin of the new reassortant virus [11,12]. Finally, and perhaps more important, it is worrying that the majority of countries suffering from human cases of avian influenza have a limited access to genetic surveillance, even though this method is very effective to detect exotic and highly divergent circulating between humans, wild and farm animals. We wish that this work promotes new discussions between policy makers and health surveillance organizations to improve current methodologies and regulations.

### Additional material

**Additional file 1: Additional table** Cumulative number of A(H5N1) hemagglutinin genes sequenced in Asian and African countries and reported to influenza surveillance networks between 2003 and 2011.

### Abbreviations

WHO: World Health Organization; GAR: Global Alert and Response; HA: Hemagglutinin; NIAID: National Institute of Allergy and Infectious Diseases; JCVI: The J. Craig Venter Institute; NCBI: The National Center for Biotechnology Information.

### Acknowledgements and funding

This work was supported (or partially supported) by the program UNAM-DGAPA-PAPIIT, project number IN201110. Also, we thank the World Health Organization Press, Office of Permissions Management and Reprint Rights for granting permission to extract and reproduce data from the Global Alert and Response. Also, we thank Dr. Irene Hanning-Jarquin for comments on the manuscript.

### Author details

1. Departamento de Medicina y Zootecnia de Aves. Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México. Ciudad Universitaria, Coyocacán, DF 04510, Mexico.  
2. NIH Chemical Genomics Center, National Human Genome Research Institute, NIH, 9800 Medical Center Drive, Rockville, MD 20850, USA.  
3. Dept. Pathology and Immunology, Washington University School of Medicine, 801 S. Euclid Ave, St. Louis, MO 63110, USA.

### Authors’ contributions

GMN, ME, MUE and MSAR designed research and performed research; GMN, ME and MSAR wrote the paper. All authors read and approved the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

### Received

31 August 2011 Accepted: 23 January 2012

Published: 23 January 2012

### References

1. FAO_Avian_Influenza: The fight against HPAI in Cambodia continues. The Food and Agriculture Organization 2011 [http://www.fao.org/avianflu/en/news/cambodia_hpai.html].

2. WHO_Avian_Influenza: Global Alert and Response-Avian Influenza.[http://www.who.int/csr/disease/avian_influenza/en/index.html].

3. Gibbs SE. Avian biology, the human influence on global avian influenza transmission, and performing surveillance in wild birds. Ann Hlth Res Rev 2010, 11(1):35-41.

4. FAO_Avian_Influenza: The Food and Agriculture Organization, Strategy and Policy documents.[http://www.fao.org/avianflu/en/strategydocs.html].

5. Charlton B, Crossley B, Hietala S: Conventional and future diagnostics for avian influenza. Comp Immunol Microbial Infect Dis 2009, 32(4):341-350.

6. WHO/OIE/FAO_H5N1_Evolution_Working_Group: Toward a unified nomenclature system for highly pathogenic avian influenza virus (H5N1). Emerg Infect Dis 2008, 14(7):e1.

7. WHO_Influenza_WHO Global Influenza Virological Surveillance.[http://www.who.int/influenza/virus/lsigis/laboratory/en/].

8. WHO: WHO global technical consultation: global standards and tools for influenza surveillance. 2011 [http://www.who.int/influenza/resources/documents/technical_consultation/en/index.html].

9. Briand S, Mounts A, Chamberland M: Challenges of global surveillance during an influenza pandemic. Public Health 2011, 125(3):247-256.

10. Smith GL, Fan XH, Wang J, Li KS, Qin K, Zhang JX, Vijaykrishna D, Cheung CL, Huang K, Rayner JM, et al Emergence and predominance of an H5N1 influenza variant in China. Proc Natl Acad Sci USA 2006, 103(45):16936-16941.

11. Smith GL, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, Ma SK, Cheung CL, Raghwani J, Bhatt S, et al Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza a epidemic. Nature 2009, 459(7250):1122-1125.

12. Nava GM, Attene-Ramos MS, Ang JK, Escoria M: Origins of the new influenza A(H1N1) virus: time to take action. Euro Surveill 2009, 14(22):19228.

13. Escoria M, Camillo-Sanchez K, March-Mifsut S, Chapa J, Lucio E, Nava GM: Impact of antigenic and genetic drift on the serologic surveillance of H5N2 avian influenza viruses. BMC Vet Res 2010, 6:57.

14. Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A, Sessions WM, Xu X, Skepner E, Deyde V, et al Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science 2009, 325(5937):197-201.

15. WHO_Avian_Influenza: Cumulative Number of Confirmed Human Cases of Avian Influenza A/H5N1 (H5N1) Reported to WHO.[http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/index.html].

16. Global_Alters_and_Response: Alert and response operations. [http://www.who.int/csr/alertresponse/en/].

17. WHO_Avian_Influenza: Collecting, preserving and shipping specimens for the diagnosis of avian influenza A(H5N1) virus infection. Guide for field operations.[http://www.who.int/csr/resources/publications/surveillance/WHO_CDS_EPR_ARO_2006_1/en/index.html].

18. Bao Y, Bolotov P, Demovoy D, Kryutin B, Zaslavsky L, Tatusova T, Ostpell J, Lipman D: The influenza virus resource at the National Center for Biotechnology Information. J Virol 2008, 82(21):1196-1201.

19. Goldstein ML, Morris SA, Yena GG: Problems with fitting to the power-law distribution. Eur Phys J B 2004, 41:255-258.
20. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004, 32(5):1792-1797.
21. Rzhetsky A, Nei M. Theoretical foundation of the minimum-evolution method of phylogenetic inference. *Mol Biol Evol* 1993, 10(5):1073-1093.
22. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007, 24(8):1596-1599.
23. Gouy M, Guindon S, Gascuel O. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 2010, 27(2):221-224.
24. Wu B, Wang C, Dong G, Guo Y, Nolte OL, Deliberto TJ, Xu J, Duan M, He H. New evidence suggests Southern China as a common source of multiple clusters of highly pathogenic H5N1 avian influenza virus. *J Infect Dis* 2010, 202(3):452-458.
25. Escorcia M, Vazquez L, Mendez ST, Rodriguez-Roponi A, Lucio E, Nava GM. Avian influenza: genetic evolution under vaccination pressure. *Virology* 2008, 5:15.
26. Vijaykrishna D, Poon LL, Zhu HC, Ma SK, Li OT, Cheung CL, Smith GJ, Peiris JS, Guan Y. Reassortment of pandemic H1N1/2009 influenza a virus in swine. *Science* 2010, 328(5985):1529.
27. Li Y, Lin Z, Shi J, Qi G, Deng G, Li Z, Wang X, Tian G, Chen H. Detection of Hong Kong 97-like H5N1 influenza viruses from eggs of Vietnamese waterfowl. *Arch Virol* 2006, 151(8):1615-1624.
28. Nguyen T, Davis CT, Stembridge W, Shu B, Balish A, Inui K, Do HT, Ngo HT, Wan XF, McCarron M, et al. Characterization of a highly pathogenic avian influenza H5N1 virus sublineage in poultry seized at ports of entry into Vietnam. *Virology* 2009, 387(2):250-256.
29. Coker RJ, Hunter BM, Rudge JW, Liverani M, Hanvoravongchai P. Emerging infectious diseases in southeast Asia: regional challenges to control. *Lancet* 2011, 377(9765):599-609.
30. Davis CT, Balish AL, O'Neill E, Nguyen CV, Cox NJ, Xiyuan X, Klimov A, Nguyen T, Donis RO. Detection and characterization of clade 7 high pathogenicity avian influenza H5N1 viruses in chickens seized at ports of entry and live poultry markets in Vietnam. *Avian Dis* 54(1 Suppl):307-312.
31. LeQM, Ito M, Muramoto Y, Hoang PV, Vuong CD, Sakai-Tagawa Y, Kiso M, Ozawa M, Takano R, Kawaoka Y. Pathogenicity of highly pathogenic avian H5N1 influenza a viruses isolated from humans between 2003 and 2008 in northern Vietnam. *J Gen Virol* 91(Pt 10):2485-2490.

doi:10.1186/1756-0500-5-62
Cite this article as: Escorcia et al.: Improving global influenza surveillance: trends of A(H5N1) virus in Africa and Asia. *BMC Research Notes* 2012 5:62.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit