Vaccine 35 (2017) 1104–1109

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

WHO Report

Improving the selection and development of influenza vaccine viruses – Report of a WHO informal consultation on improving influenza vaccine virus selection, Hong Kong SAR, China, 18–20 November 2015

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ARTICLE INFO

Article history:
Received 13 December 2016
Accepted 10 January 2017
Available online 25 January 2017

Keywords:
Influenza vaccines
Surveillance
Antigenic drift
Pandemic influenza

ABSTRACT

Since 2010 the WHO has held a series of informal consultations to explore ways of improving the currently highly complex and time-pressured influenza vaccine virus selection and development process. In November 2015 experts from around the world met to review the current status of efforts in this field.

Discussion topics included strengthening influenza surveillance activities to increase the availability of candidate vaccine viruses and improve the extent, timeliness and quality of surveillance data. Consideration was also given to the development and potential application of newer laboratory assays to better characterize candidate vaccine viruses, the potential importance of antibodies directed against influenza virus neuraminidase, and the role of vaccine effectiveness studies. Advances in next generation sequencing and whole genome sequencing of influenza viruses were also discussed, along with associated developments in synthetic genomics technologies, evolutionary analysis and predictive mathematical modelling.

Discussions were also held on the late emergence of an antigenic variant influenza A(H3N2) virus in mid-2014 that could not be incorporated in time into the 2014–15 northern hemisphere vaccine. There was broad recognition that given the current highly constrained influenza vaccine development and production timeline it would remain impossible to incorporate any variant virus which emerged significantly long after the relevant WHO biannual influenza vaccine composition meetings. Discussions were also held on the development of pandemic and broadly protective vaccines, and on associated regulatory and manufacturing requirements and constraints.

With increasing awareness of the health and economic burdens caused by seasonal influenza, the ever-present threat posed by zoonotic influenza viruses, and the significant impact of the 2014–15 northern hemisphere seasonal influenza vaccine mismatch, this consultation provided a very timely opportunity

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http://dx.doi.org/10.1016/j.vaccine.2017.01.018
0264-410X/

to share developments and exchange views. In all areas, a renewed and strengthened emphasis was placed on developing concrete and measurable actions and identifying the key stakeholders responsible for their implementation.

1. Introduction

Efforts to establish a global network for detecting and identifying new and potentially dangerous influenza viruses predate the adoption of the WHO Constitution in 1948. Today, the WHO Global Influenza Surveillance and Response System (GISRS)[1] serves as the worldwide coordination mechanism for monitoring and responding to the threats posed by influenza viruses. GISRS also facilitates the use of the most epidemiologically and geographically relevant vaccine formulations. Since 1998, separate and appropriately timed vaccine virus recommendations have been issued following the WHO vaccine composition meetings (VCMs) held each year in February and September for the northern and southern hemispheres respectively.

Retrospective studies have shown that these WHO recommendations have resulted in a relatively acceptable antigenic correspondence (match) between the viruses used in the vaccines and influenza viruses circulating during the following influenza season. Nevertheless, various degrees of antigenic divergence (mismatch) between vaccine and circulating viruses have occurred due to ongoing antigenic variation, including the unpredictable and "late" emergence of a significant antigenic variant virus following the biannual WHO VCMs, and due to issues related to egg adaptation during vaccine production. Historically, significant vaccine mismatches occurred in both the 1997–98 and 2014–15 (northern hemisphere) influenza seasons due to the late emergence of variant viruses, and in the 2003–04 season due to the lack of a suitable high-growth vaccine candidate for emergent A/Fujian/411/2002-like viruses.

Such events highlight the complexity of the influenza vaccine virus selection, development and production process. Despite significant scientific, technical and manufacturing advances, and greatly improved understanding of influenza virus evolution, the timeline for completing this process has remained largely unchanged for decades. Current vaccine technologies and production schedules mean that decisions on vaccine composition have to be made almost a full year in advance of the peak of seasonal influenza activity. As a result, the system remains severely time constrained and the detection of a newly emerging and antigenically distinct variant in April–May – as occurred in 2014–15 [1] – provides insufficient time to produce an updated vaccine for the upcoming northern hemisphere influenza season.

In order to address the recognized severe time constraints and other challenges inherent in the current system WHO has since 2010 held a series of regular informal consultations. These consultations [2–4] have explored in detail a range of activities aimed at improving the influenza vaccine virus selection and development process by providing a platform for expert discussion across a broad range of key areas. In November 2015 the fourth informal consultation brought together experts from around the world to review the current status of efforts in the field. Particular attention was given to the following areas:

- strengthening influenza surveillance activities to improve the availability of candidate vaccine viruses;
- improving the virological characterization and evaluation of candidate vaccine viruses;
- applying next generation sequencing (NGS) technology;
- the potential role of enhanced evolutionary analysis and predictive modelling;
- the development of pandemic and broadly protective ("universal") vaccines; and
- addressing regulatory requirements for influenza vaccines.

In all these areas, a renewed and strengthened emphasis was placed on developing concrete and measurable actions, and on identifying the key stakeholders responsible for their near- and longer-term implementation.

2. Strengthening influenza surveillance activities to improve the availability of candidate vaccine viruses

In recent decades the number of WHO Member States participating in GISRS has steadily increased, resulting in significant surveillance gains, particularly in the aftermath of the 2009 H1N1 pandemic. However, there remains a need for improved influenza monitoring and reporting, and for the more-timely and routine sharing of influenza viruses, especially by countries in currently under-represented regions of the world. In addition, there is an absence of national influenza vaccination policies in many tropical and subtropical countries, affecting around 60% of the world's population. Even where vaccines are used in such regions of the world there is often a lack of clarity regarding the basis upon which vaccines are selected for national programmes and a lack of surveillance and other data to support vaccination composition and timing decisions.

Meeting participants were informed of recent WHO efforts to develop guidance and provide support to tropical and subtropical countries in introducing seasonal influenza vaccination policies and programmes. During a series of WHO meetings, independent multi-agency evaluations of influenza seasonality in the tropics and subtropics have provided the basis of discussions on the development of national roadmaps and evidence-based approaches for influenza vaccine introduction, and for the development and implementation of a package of WHO guidance and recommendations in this area [5–7]. Specific issues covered included determining which of the two annual influenza vaccine compositions (northern or southern hemisphere) to use and how to establish the optimal timing of vaccination.

Although national, regional and global influenza surveillance capacity has been strengthened in recent years much of this has focused on ensuring the detection of viruses with pandemic potential. There is thus a need for a systematic WHO review of seasonal influenza surveillance capacities and capabilities. Such a review could then be used to inform a process of national capacity-building for influenza surveillance within GISRS, particularly in currently under-represented countries and regions.

Key actions proposed in this area included: (a) WHO to continue to promote global national influenza centre (NIC) capacity-building by communicating to national authorities the vital public health contributions made by NICs, and the need for continued and enhanced support for their activities; (b) reviewing the current global influenza surveillance landscape and approaches to promote the optimal use of available resources and identify opportunities for strengthening influenza epidemiological and laboratory

[1] Formerly known as the Global Influenza Surveillance Network prior to the adoption of the World Health Assembly Resolution WHA 64.5 on 24 May 2011. As of September 2015 GISRS consisted of 143 National Influenza Centres (NICs) in 113 countries, six WHO Collaborating Centres (WHOCCs), 13 WHO H5 Reference Laboratories and four WHO Essential Regulatory Laboratories (ERLs).

capacities, particularly in under-represented world regions; (c) providing support to national and regional efforts to improve the collection, analysis and use of influenza surveillance and burden-of-disease data in tropical and subtropical countries; (d) providing revised detailed operational and other guidance to NICs in areas such as virus sampling and selection strategies, the reporting of virus activity, the timely provision of genetic data to publicly accessible databases, and the best practicable frequency of virus sharing; and (e) further strengthening of WHO technical, logistical and other assistance to NICs across the broad range of sampling, reporting, shipping and related activities required to ensure the timely sharing of information and representative viruses.

3. Improving the virological characterization and evaluation of candidate vaccine viruses

For over 60 years the antigenic characterization of influenza viruses (and thus vaccine virus selection) has traditionally relied upon the use of the haemagglutination inhibition (HI) assay to evaluate the ability of specific antibodies to inhibit the binding of virus haemagglutinin (HA) to red blood cell (RBC) receptors. As a surrogate for virus neutralization, the HI assay is routinely used to inform the biannual WHO recommendations on influenza vaccine composition. However, following its emergence in 1968, the current influenza A(H3N2) virus subtype evolved over the period 1990–2005 to progressively display reduced avidity for chicken, turkey and guinea-pig RBC receptors, with the resulting loss of binding to all but the latter type of RBC [8]. Due to their reduced receptor binding to MDCK cells, these A(H3N2) viruses acquire amino acid substitutions at residues 148 and 151 of the neuraminidase (NA) during cultivation resulting in RBC binding by the NA component. This then necessitates the addition of the antiviral oseltamivir carboxylate to the HI assay to exclude this confounding effect [9].

During 2013–14, further evolution in the HA resulted in some MDCK-cell propagated viruses failing to agglutinate even guinea-pig RBCs, impacting upon both the detection and antigenic analysis by HI assay of the seasonal A(H3N2) viruses. This was the case for the majority of viruses in the H3 clade 3C.2a that emerged during 2014–15. In addition, it has long been recognized that the traditional propagation of influenza viruses in embryonated chicken eggs can select for HA sequence changes that influence antigenicity. This complicates vaccine composition decisions, particularly in relation to recent A(H3N2) viruses.

In response to these and other developments, virus neutralization (VN) assays that directly detect antibodies that prevent cell infection have increasingly been used to complement HI antigenic analysis using the same reference ferret antisera, and to more sensitively measure human antibody titres, particularly at the threshold of detection. In addition, accumulating experimental evidence indicates that antibodies directed against influenza virus NA can also confer a degree of protection from illness [10]. For example, individuals with previously acquired NA antibodies that were cross reactive with either the 1968 A(H3N2) or 2009 A(H1N1) pandemic viruses were found to be less likely to be infected or to suffer infection-related illness during these pandemics [11,12]. An enzyme-linked lectin assay for quantitating antibodies against NA has now been evaluated and shown, through the CONSISE consortium,[2] to exhibit good consistency when used in conjunction with an antibody reference preparation [13].

Vaccine effectiveness (VE) studies also represent another potential approach to the strengthening of vaccine virus selection. The recently developed "test-negative" design has now been widely adopted for VE estimations in which a sample of patients presenting with influenza-like illness are tested for influenza and a comparison made between the odds of vaccination among those with laboratory-confirmed influenza infection and the odds of vaccination among those who test negative [14]. Obtaining robust VE estimates requires the collecting of sufficient numbers of samples in each category. Given sufficient sample sizes, the method can also provide VE estimates for each of the different influenza types/subtypes and lineages represented in a multivalent vaccine. The results of such studies can provide a retrospective assessment of the vaccine virus selection process and highlight the need for any updating of individual vaccine virus components.

Proposed actions in this area included: (a) identifying additional sources of human pre-/post-vaccination human serum panels to better characterize human antibody responses to newly circulating viruses; (b) creating a VN working group to optimize and harmonize protocols and testing strategies based upon recent advances in this technology; (c) establish a broadly based interest group to address the feasibility of NA as a potentially quantified vaccine antigen, particularly as next-generation recombinant vaccines are developed; and (d) harmonize VE protocols among all the current different studies/sites to allow for comparable and more robust analyses.

4. Applying next generation sequencing (NGS) technology

NGS is a powerful tool with a broad range of current and potential future applications in influenza virus surveillance, candidate vaccine virus selection and other vaccine development activities. Key benefits of using sequence data include the ease with which direct comparisons of gene sequences of different viruses can be made, as opposed to HI data comparisons of different viruses conducted at different times which require algorithms and expertise (antigenic cartography). Furthermore, where feasible, the provision of sequence data by NICs at the time of sample shipment to WHO Collaborating Centres (WHOCCs) would facilitate the selection of viruses for egg or cell culture isolation. Such early availability of sequence information either from NICs directly or through the prompt sequencing of original clinical specimens forwarded to WHOCCs may also potentially shorten the timeline for deriving and prioritizing suitable egg isolates and reassortants for use as candidate vaccine viruses, which currently takes at least 3–4 months.

Activities are thus continuing in a range of countries, agencies and GISRS laboratories in relation to an anticipated paradigm shift in influenza surveillance and related processes associated with the introduction of NGS and whole genome sequencing (WGS) of influenza viruses. Achieving the economy-of-scale and related benefits of NGS is, however, also accompanied by the demands of initial sample preparation, bioinformatics capabilities, and "big data" storage and analysis which are currently rate-limiting steps. Associated challenges include the need for adequate viral loads in original specimens, preventing interference by host and other pathogen genes, and avoiding biases in coverage and sampling. Improving capability will thus require addressing issues such as sampling and operating protocols, data quality, process efficiency, workforce planning, automation, and the handling, analysis, storage and timely sharing of sequence data and linked metadata. Corresponding efforts will also be required to preserve laboratory virus-culture capabilities at the national level, to right-size sampling of specimens for testing, and to improve culture systems. The increased use of sequence data to prioritize the phenotypic

[2] Following its establishment in 2011, the Consortium for the Standardization of Influenza Seroepidemiology (CONSISE) has worked to standardize the seroepidemiology of influenza and other respiratory pathogens, and to develop comprehensive investigation protocols for use in responding to both seasonal and potentially pandemic influenza viruses, and other respiratory pathogens. See https://consise.tghn.org/.

characterization of viruses also has the potential to improve data timeliness and availability during the biannual WHO VCMs. Current constraints include the need to conduct VCMs before the end of the season (with inadequate opportunity for HI or VN analysis of samples received in the preceding 3 weeks) and delays in virus shipment to GISRS reference laboratories.

As with other aspects of influenza surveillance (see above) the concept of capacity-building in NICs and other GISRS laboratories provides an overarching approach for systematically putting in place the range of capabilities needed to realize the gains of a shift towards WGS and NGS. Such an approach could be greatly facilitated through strengthened engagement with the global donor community and harnessing of the capacity-building components of major international health agreements such as the Pandemic Influenza Preparedness (PIP) Framework for the sharing of influenza viruses and access to vaccines and other benefits (www.who.int/influenza/pip/en/). Potential key steps by countries in this area include implementing broader and more integrated national approaches to respiratory and other disease surveillance involving other public health needs.

Specific proposed actions in this area included: (a) conducting a survey of current Sanger and NGS sequencing capabilities and activities undertaken by NICs which incorporates an evaluation of the needs of laboratories in this area; (b) documenting the range of current capacity-building opportunities and potential synergies for expanding the use of NGS within GISRS, including provision of training and guidance to NICs in aspects such as platform selection, large-scale computerized data storage, and data analysis and reporting; (c) developing revised and strengthened guidance for NICs on the required characteristics and optimal extent of sequence and antigenic data, and on the key need to ensure the timely submission of sequence data and virus samples to WHOCCs; (d) identifying approaches for capturing genetic polymorphisms in NGS databases, and for the routine retrieval of such data; and (e) working with the animal health sector on cross-cutting issues such as quality assurance standards, data storage, analysis and management, sharing of bioinformatics pipelines and capacity-building.

5. Potential role of enhanced evolutionary analysis and predictive modelling

Complex mathematical modelling and associated techniques are increasingly being used to gain insights into the evolution and epidemiology of influenza viruses. Such techniques include, for example, the integration of sequence data with other virus characteristics such as epidemiology and geographical location [15], the use of the concept of viral fitness (defined as the expected growth rate of virus clades) as a potential predictor of virus evolution [16], and the use of continuously updated phylogenetic databases to predict the growth and decline of virus clades [17]. Investigations have also been carried out into the utility of generating potential ‘‘antigenically advanced” viruses by inducing random mutations in the HA gene region coding for the globular head of the HA protein. Following the compiling of a mutant virus library using reverse genetics, viruses with the potential to escape existing immunity can then be detected using ferret or human antibodies directed towards the parental virus [18].

The application of non-mechanistic statistical algorithms, such as those already used as the basis of antigenic cartography have already proved useful in facilitating vaccine virus selection, and in aiding assessment of the pandemic potential of avian and other animal influenza viruses. Complex modelling based on modified antigenic cartography can reduce the ‘‘noise” caused by variable serum quality and potency, and has the potential to combine antigenic and phylogenetic data into a single evolutionary analysis that quantifies both the antigenic and evolutionary distances between strains. Such approaches have shown, for example, that the antigenic drift of seasonal influenza A(H3N2) is more rapid than that of seasonal A(H1N1), followed by the type B Victoria lineage, with the B/Yamagata lineage being the slowest, with all drift approximately occurring in proportion to the rate of genetic change [19].

Actions proposed to further explore the applicability of these and related approaches included: (a) organizing a meeting to broaden and strengthen mutual understanding of all the different modelling systems between the modellers themselves and WHOCCs, and to assess how best to evaluate and harness the potential season-by-season contributions of each system; and (b) developing a route for the more formalized incorporation of modelling data and findings into the considerations of the biannual WHO VCMs.

6. Development of pandemic and broadly protective (‘‘universal) vaccines

A number of significant challenges and fundamental gaps remain to be addressed in determining optimal approaches to pandemic vaccine preparedness. Despite the possibility that the degree of cross reactivity of antibodies generated by current candidate pandemic vaccine viruses in humans may be broader than that previously indicated by ferret sera studies, there is a lack of data on the precise nature of responses in humans. In light of the substantial development and other costs associated with candidate pandemic vaccine viruses, and an ever-increasing list of H5 candidate viruses based on reactions with ferret sera, there is now a need for more human serology data from H5 vaccine recipients to more accurately assess the number of such viruses required. There is also a need for continuing and strengthened collaboration between the human and animal influenza surveillance sectors, particularly with respect to those animal subtypes not currently associated with human infection, and for a systematic approach to reagent development. Currently only subtypes responsible for human infections are subject to review at the biannual WHO VCMs. Serious constraints also persist on the distribution of reagents for candidate pandemic viruses with a variety of different national shipping laws and restrictions involving departments of health, agriculture and commerce needing to be met and, for many viruses, a requirement for high-containment laboratories.

Recent zoonotic infections and the ever-present threat of a pandemic have also led to a massive increase over the last decade in the volume of scientific papers relating to the development of broadly protective (‘‘universal”) influenza vaccines; illustrating the high level of interest in this area. In addition, as highlighted above, there is a continuing risk of the unavoidable late emergence of a virus variant during the current seasonal influenza vaccine production cycle. A number of studies have focused on the potential role of either cross-reactive antibodies or T-cell immunity [20]. Potential viral targets for cross-reactive antibodies include the M2 protein and the stalk region of the HA protein, while targets for cross-reactive T-cell immunity include the structural proteins, particularly NP and M1, non-structural proteins and polymerase proteins. Recent animal-model and human studies have therefore involved the investigation of a range of potential approaches, including cytotoxic T-cell (CTL) immunity [21], M2 protein ectodomain (M2e)-specific antibodies [22] and HA stalk region antibodies [23,24]. The further development of broadly protective vaccines will require human immunogenicity and protection studies, determination of correlates of protection, establishment of safety and confirmation that the targeted sites remain conserved under vaccine-induced immune pressure.

Proposed actions included: (a) reiterating to NICs, as outlined in the PIP Framework, the need to promptly share with WHOCCs all

viruses, clinical samples and information relating to all cases of human zoonotic infections; and (b) reviewing the evidence base to support the case for reduced animal pathogenicity testing of candidate pandemic vaccine viruses derived using reverse genetics and intended for use against highly pathogenic avian influenza viruses.

7. Addressing regulatory requirements for influenza vaccines

Conventionally developed candidate vaccine viruses are subjected to a broad range of tests prior to use, including identification of the HA and NA type/subtype and lineage of the component viruses, and of the degree to which each component virus antigenically accords with the corresponding circulating virus "prototype". Other tests include full genotyping, HA/NA gene sequencing, absence of parental high-yield PR8 HA, antigenicity in a two-way HI test, HA titre and infectivity. Estimates of yield are also considered desirable by manufacturers. For the quality control of both seasonal and potential pandemic vaccines, the preparation and standardization of currently accepted potency reference reagents for inactivated influenza vaccines can represent a significant bottleneck in the timeline for vaccine availability. In particular, the shipping and sharing of reagents for candidate pandemic vaccine viruses can be subject to high-containment laboratory requirements, thus accentuating the problem. Because vaccine manufacturer seed laboratories are typically limited to BSL-2 enhanced containment, and global vaccine manufacturing capacity meets BSL-2 enhanced containment, manufacturers are highly dependent upon rapid safety testing and clear communication of the outcome when seeking to commence work on pandemic or potential pandemic candidate vaccine viruses. In addition, candidate vaccine viruses prepared by reverse genetics are considered to constitute genetically modified organisms (GMO) in some countries and as a result are subjected to further national regulations and restrictions.

In light of the current constraints in both seasonal and pandemic vaccine production there is industry support for: (a) the development of alternative potency assays; (b) convergence of national regulatory authority evaluations needed to ensure the quality, safety and efficacy of vaccines; (c) establishment of a mutual recognition system for pandemic vaccine registration; and (d) use of international WHO package labelling to harmonize prescription guidance and avoid delays in pandemic vaccine deployment.

An opportunity exists to review these and related issues as part of a broader exploration of the current landscape of candidate vaccine virus development, including evaluation of prevailing global regulatory opinions, resource-prioritization strategies and associated aspects. Such an exploration might now be beneficially informed by a systematic comparative review of the potential advantages and safety of viruses generated by reverse genetics compared to conventionally generated viruses.

Specific proposed actions included: (a) reviewing and comparing existing data on the time required to generate candidate vaccine viruses (optimized for growth in eggs and/or cells, and with acceptable antigenicity) by reverse genetics or by classical reassortment; and (b) making the initial scientific case for the equivalence of candidate vaccine viruses generated by reverse genetics and those generated by classical reassortment with respect to GMO status, as part of a process of early case-building and advocacy.

8. Conclusion

Against a backdrop of increasing awareness of the health and economic burdens caused by seasonal influenza, the ever-present threat posed by zoonotic influenza viruses and the significant 2014–15 northern hemisphere seasonal influenza vaccine mismatch this consultation provided a particularly timely opportunity to share recent developments in the field and to exchange views via both panel-based and plenary discussions. With a greater emphasis now placed on ensuring measurable progress through feasible and coherent actions, efforts will now be accelerated to progress all of the near- and mid-term goals identified.

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