Toward standardized influenza seroepidemiology – 4th international CONSISE meeting

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Synopsis:
The report summarizes the main conclusions of the 4th international Consortium for Standardization of Influenza Seroepidemiology (CONSISE) meeting, held 3-4 September 2013, Cape Town, South Africa. The workshop brought together influenza epidemiologists, virologists and public health experts to discuss the standardization of laboratory and epidemiology methods and protocols for influenza and other respiratory virus serology.

Background

The Consortium for Standardization of Influenza Seroepidemiology (CONSISE) (1, 2) held its 4th international meeting in Cape Town in September 2013. CONSISE is composed of globally recognized experts and institutes who are interested in the standardization of seroepidemiology for influenza and other respiratory pathogens. There are currently more than one hundred members of CONSISE based in more than 45 countries. CONSISE has two integrated working groups, one for epidemiology and one for laboratory issues. CONSISE was formed after the 2009 influenza pandemic when it was recognized that there was a need to provide more timely and standardized influenza seroepidemiology results to inform decision making (1, 2). Background information and the organization of CONSISE can be found at http://consise.tghn.org/about/.

Within the two-and-half years of CONSISE's existence, the epidemiology and laboratory working groups have made substantial progress which was reviewed at the meeting in Cape Town.

Work Plan and Achievements

Epidemiology working group

One of the overall aims of CONSISE is to recommend best practices and standardize influenza seroepidemiology studies for pandemic, epidemic, zoonotic influenza viruses and emerging respiratory viruses. By doing so, we aim to provide support to organizations who wish to carry out seroepidemiology studies for, primarily, influenza, but also other emerging respiratory pathogens, by providing: (1) tools in the form of protocol templates and questionnaires; (2) recommendations on which study to conduct when; (3) guidance on adaptation of tools to meet specific objectives and contexts; (4) guidance and support of implementation of seroepidemiologic studies; (5) coordination of laboratory support, if capacity does not exist in country; and (6) analysis and writing up of findings, when requested.

The main task of the epidemiology working group is to generate detailed study protocol templates that can be used to evaluate the seroprevalence of seasonal, pandemic and zoonotic influenza viruses in specific populations. The templates will generate synergy and comparability between studies. We aim to generate these protocol templates mainly for influenza, but also in a way that they can be adapted for other respiratory pathogens, such as the novel coronavirus identified in the Middle East in 2012 (MERS-CoV).

The epidemiology working group has drafted seven influenza seroepidemiology protocol templates of different study designs. These include: prospective longitudinal cohort for pandemic influenza, serial cross-sectional seroepidemiological investigation, household transmission study, closed setting transmission study, routine/residual sera, close contact serologic investigation for zoonotic influenza and assessment of health care workers (2). Templates have been developed based on detailed protocols used in previous seroepidemiological studies in a range of situations across the globe. Generic parts and lessons learned have been incorporated in the templates for future use. Three protocol templates are now openly available on the CONSISE website

(http://consise.tghn.org/articles/available-consise-influenza-protocols/). The remaining four protocols are undergoing an extensive review and will be posted in the coming months.

One of the challenges of developing questionnaires to accompany each of CONSISE's protocol templates is that it is impossible to anticipate all questions in advance as the exact epidemiologic situation and the context of the outbreak will be unknown and unique. To address these uncertainties, the working group is developing a question bank, which holds a collection of questions under major headings such as background information, medical and vaccination history, exposures (animal, environmental, occupational, etc), travel history, signs and symptoms, healthcare worker-specific questions, etc. The question bank is designed to facilitate the rapid development of questionnaires in conjunction with the study protocols. An online interface will be developed for users to download specific questions for their own questionnaires.

In recent months, several of CONSISE's protocols have been adapted for MERS-CoV and for influenza A(H7N9) outbreaks in the Middle East and China, respectively. With key epidemiologic information learned from for each of these epidemics, CONSISE members in collaboration with organizations such as the World Health Organization and China Centers for Disease Control have modified the methodologies of the generic protocols to be relevant for MERS-CoV and H7N9 (http://consise.tghn.org/articles/novel-coronavirus-ncov/; http://www.who.int/csr/disease/coronavirus_infections/en/).

CONSISE is actively engaging research institutions and public health agencies in several countries to support implementation and field validation of CONSISE protocols. The feedback received will be used to improve the protocol templates.

Laboratory working group

One of the main tasks of the laboratory working group is to coordinate and standardize the international serology laboratory response to a new emerging influenza virus. Two main methods are used in influenza serology to detect influenza antibodies: haemagglutination inhibition assay (HI) and microneutralization assay (MN). Several protocols exist for each test and therefore the laboratory working group has reviewed the members' protocols for MN and HI assays. The group has developed two consensus protocols for MN and in this meeting agreed on a consensus protocol for the HI assay. A collaborative study has been undertaken to compare different MN protocols, namely the 2-day ELISA-based and the 3-day haemagglutination (HA) assay-based methods. The results of 11 laboratories using A(H1N1)pdm09 strain were reviewed in an earlier meeting and it was concluded that these two assays are comparable and therefore either of these CONSISE consensus protocols could be recommended. MN assay comparison has been extended to H3N2 and H5N1 viruses and the analysis of these results is underway. The preliminary results indicate generally good correlation between the two consensus protocols.

The CONSISE laboratory group is strongly in favor of keeping HI as the primary serology assay, but will assess how it can be better standardized. A comparative study using the agreed HI consensus protocol will be conducted during 2014. There was general agreement to look at laboratory-to-laboratory variability in the upcoming collaborative studies of both

MN and HI. For the MN inter-laboratory variability study, either 2-day or 3-day assay can be used. The study will be conducted using A(H1N1)pdm09 virus(es) and a small study group will be established to develop the detailed study protocol. A panel of human A(H1N1)pdm09 positive sera will be distributed to participating laboratories. At the same time, various sources of potential antibody standards will be evaluated.

The laboratory working group has recommended that international antibody standards are used wherever possible and reviewed possible sources of antibodies. At present these standards are human antibodies to specific strains. Possible sources include human serum or plasma from convalescent patients or post-vaccination. For an international standard preparation approximately one liter of human serum is needed. Animal sera could possibly serve as a standard in a situation where human serum is not available in sufficient amounts. The group also discussed the use of monoclonal antibodies and human antibodies produced in trans-chromosomal cows both of which could be produced more quickly and in larger quantity than convalescent human sera. It was agreed that formal approval of an antibody standard by WHO may take too long, so alternative systems should be considered.

Four of the CONSISE laboratories have implemented the enzyme-linked lectin assay (ELLA) (3) for detection of neuraminidase (NA) antibodies and a small group will plan a collaborative ELLA study. Other NA serology assays have also been assessed by the participating laboratories but some technical issues still need to be resolved.

New serology assays were also reviewed. Those include variations to existing assays, e.g. use of CaCo2 cell line instead of MDCK in MN assay or use of stabilised red blood cells (RBCs) in the HI assay. A pseudoparticle MN assay has been evaluated by one laboratory and while the results correlated with the classical MN, the pseudoparticle preparation requires further standardisation. A protein microarray assay is a quick and subtype-specific test for positive/negative serum results and has been evaluated (4). Some point-of-care tests are also under evaluation. Many of these newer tests require further validation.

CONSISE response to influenza A(H7N9)
CONSISE has convened teleconferences during the influenza A(H7N9) outbreak in China and has shared through web-posting the HI/MN assay protocols developed by China CDC for A(H7N9) . The initial HI assay protocol has been modified and improved in collaboration with the US CDC and this modified assay is recommended by CONSISE (available at http://consise.tghn.org/articles/consise-and-avian-influenza-h7n9/).
China CDC presented results of A(H7N9) serology studies in China. The findings show that although A(H7N9) can bind to both avian-type (α2, 3-linked sialic acid) and human-type (α2, 6-linked sialic acid) receptors, horse RBCs increase the sensitivity of HI in detecting antibody response to H7N9 virus compared to turkey RBCs.

CONSISE response to MERS-CoV
An overview of the early investigations of MERS-CoV from the WHO perspective was presented. The main focus was on the types of protocols needed in case of a new emerging virus: initial patient interview, case-control study of exposures, study in health care facilities, contact study, serial cross-sectional surveys of risk groups and animal surveys to identify the source of the virus. On the laboratory side, PCR is usually set up quickly and is the detection

method of first choice. Retrospective testing of stored specimens of e.g. severe acute respiratory infection cases or all unexplained pneumonia in affected countries can be useful although cost and human resources are limiting factors. Serology, as a way of identifying immunity or infection, is crucial but the limited availability of viruses and positive sera has posed a challenge. Also due to lack of standards and cross-reactivity the interpretation of the results may be difficult. However, serology is useful even if it is methodically imperfect as relative positivity of different groups can be compared. As an example, in case of MERS-CoV, seropositive results, are currently classified as "probable" cases requiring a PCR confirmation. Due to the complexity of serology and the validation of serological results, international collaboration is crucial. Several of the draft CONSISE protocol templates are undergoing further revision for MERS-CoV by WHO and will be released by WHO and CONSISE soon.

Upcoming advice from CONSISE

Criteria for sero-positivity for highly pathogenic avian influenza A(H5N1)

CONSISE members discussed the criteria for seropositivity of avian influenza A(H5N1) virus infection. The traditional criteria for seropositivity for avian influenza in humans has been: titer of ≥40 in two independent MN assays or seroconversion (≥4-fold rise) between acute/convalescent paired sera with Western blot as confirmatory assay to enhance specificity. However, H5N1 seroepidemiology studies use different assays and criteria for seropositivity, making it difficult to compare and interpret findings (5). CONSISE plans to generate a consensus statement for the recommended serologic assays, timing of sample collection and criteria for seropositivity for H5N1 seroepidemiologic studies.

Networking and fund raising

CONSISE links directly through its members with several existing networks, e.g. WHO Global Influenza Surveillance and Response System (GISRS), International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC), the Global Health Network, and European Influenza Surveillance Network (EISN). Raising awareness of the work of CONSISE is still needed and CONSISE members were urged to spread the word in any context that they see useful. CONSISE is currently not receiving funding for its activities, other than meeting support. Therefore, the CONSISE steering committee is exploring possible funding sources. The CONSISE secretariat has been managed from 2011 until end of this meeting by ECDC and was taken over by the University of Hong Kong in September 2013.

Conclusions

CONSISE has achieved a considerable amount of consensus and has taken key decisions during the 2.5 years of its existence. The group members are highly motivated and keen on achieving better results during the next outbreak or pandemic of a new influenza or other respiratory viruses. The main achievements are the consensus laboratory protocols for influenza A(H1N1)pdm09 microneutralization assays and the epidemiological template protocols for influenza and MERS-CoV posted on the CONSISE website. The epidemiological templates require further field validation and comments from the users will help to improve the protocols further. CONSISE aims for greater awareness of its work by linking to other existing networks and being in the frontline of communicating the existing products to public health experts working on respiratory illness outbreaks.

We recognize that not all research questions can be answered by serological studies alone and have recommended complementary study designs to be used simultaneously in an outbreak situation to address unknowns. CONSISE is developing mechanisms which will aid existing networks and institutes during emergent situations.

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