IVMONLINE

INFLUENZA VIRUS MONITORING

USAID | INDONESIA

FOREWORD

DIRECTOR GENERAL OF LIVESTOCK AND ANIMAL HEALTH SERVICES

By the grace of God almighty, we are grateful that the Influenza Virus Monitoring (IVM) platform **IVM Online** could be realized. The IVM programme was established through collaboration between the Government of Indonesia and the Food and Agriculture Organization. This programme is implemented by all technical implementing units across the Directorate General of Livestock and Animal Health Services and will be further developed, where in the future it could involve the private sector or universities. It is expected that through the **IVM Online** platform the mapping of avian influenza viruses in Indonesia can be done to allow policy making in the control and prevention of avian influenza.

Thank you to all parties who gave contributions, ideas, or played active roles in the development of the **IVM Online** programme. It is expected that this programme can be utilized optimally for the control and prevention of avian influenza and other epidemic and zoonotic influenzas in Indonesia.

Ir. Syukur Iwantoro
Director General of Livestock and Animal Health Services

DIRECTOR OF ANIMAL HEALTH

IVM Online is a new system in animal disease monitoring. The existence of influenza viruses, in particular HPAI virus in Indonesia, raises concerns on the development of zoonoses in humans. Therefore, **IVM Online** is one of the systems used to manage and monitor AI virus in an integrated manner. The **IVM Online** programme is implemented within a network to allow data generation anywhere, in a faster and real-time manner.

IVM Online is significantly useful in the improvement of laboratory capacity to support animal health. It is expected that this programme could support the establishment of a veterinary laboratory network across Indonesia. In the future, it is expected that private laboratories as well as universities could join and participate in **IVM Online**.

Dr. Pudjiatmoko
Director of Animal Health

FAO REPRESENTATIVE - INDONESIA

The launch of the Influenza Virus Monitoring (IVM) platform **IVM Online** marks a milestone in the continuing collaboration between the Directorate General of Livestock and Animal Health Services (DGLAHS), Ministry of Agriculture and FAO Indonesia. This partnership has flourished since 2006 through a series of technical projects supporting the control of highly pathogenic avian influenza (HPAI). In efforts to control HPAI and reduce the threat to human health, as well as the economic losses to the national economy and to the poultry sector, Indonesia has used vaccination as the principal means of controlling the disease. During this time, the virus has undergone gradual antigenic drift, which has necessitated changes in seed strains for vaccine production and associated modifications to diagnostic reagents. In order to improve the system of monitoring such viral evolution, the Government of Indonesia, with the assistance of FAO/OFFLU and regional reference laboratories such as the Australian Animal Health Laboratory, has developed an innovative IVM network whereby H5N1 isolates are antigenically and genetically characterized.

The benefits of the IVM network approach to HPAI surveillance was demonstrated by the detection of a new clade ("2.3.2.1") H5N1 virus in Indonesia in 2012. The new virus, which caused high mortality in domestic ducks, was isolated by the Wates DIC, and then sequenced by the IVM partner laboratories. The resulting antigenic and genetic characterization of this new clade virus lead to the successful and timely development (by Pusvetma) of a clade 2.3.2.1 vaccine ("Afluvet"), based on the Sukoharjo strain.

The example of the Indonesian IVM network, and the development of the **IVM Online** platform have relevance for other countries in the Asia region seeking to establish laboratory networks for the surveillance of avian influenza and other pathogens. FAO looks forward to a continuing partnership with the DGLAHS in the prevention and control of emerging infectious diseases of veterinary and public health significance.

Dr. Mustafa Imir
FAO Representative
Indonesia
20 May 2014

THE EFFORT TO FIGHT INFLUENZA VIRUS IN INDONESIA

The poultry industry makes up 1% of Indonesia's gross domestic product and provides the protein needs of 232 million Indonesians. The complex poultry industry structure, starting from intensive poultry farms, semi-intensive broiler and layer farms, and small scale backyard poultry farms provide meat and eggs through traditional markets all over Indonesia. The introduction of the H5N1 Highly Pathogenic Avian Influenza (HPAI) virus in 2003 has disrupted the productivity of the poultry industry. Vaccination is used as one of the 8 strategies to fight the influenza virus in poultry.

Since 2009, the Government of Indonesia, supported by the influenza expert network of OIE/FAO (OFFLU) and FAO – ECTAD Indonesia have tried to increase the capacity of eight (8) animal health diagnostic laboratories, PUSVETMA, BBLITVET and BBPMSOH to detect and monitor the presence of the avian influenza virus circulating in Indonesia. The laboratory network was then developed to identify potential virus variants; determining the candidate strains for challenge virus and monitoring the efficacy of AI vaccines used. The diagnostic methods are harmonized with standard biological reagents for high quality results. The characterization of clade 2.1.3 H5N1 AI in the laboratory provided the following results:

A. Determine 4 vaccine strains:
    1. A/Chicken/West Java/PWT-WIJ/2006;
    2. A/Chicken/Pekalongan/BBVW-208/2007;
    3. A/Chicken/Garut/BBVW-223/2007;
    4. A/Chicken/West Java (Nagrak) 30/2007

   and 2 challenge strains:
    1. A/Chicken/West Java-Subang/29/2007 dan
    2. A/Chicken/West Java/Smi-Pat/2006 through the Director General of Livestock and Animal Health Service's decision letter on the use of local vaccine that matches the virus epidemiologically.

B. Detect the new clade 2.3.2.1 H5N1 HPAI virus in mid-August 2012 that infects ducks and has spread to islands outside of Java *(see Figure 1)*. With the presence of two H5N1 HPAI clades, the HPAI situation in Indonesia becomes more complex and requires a rapid communication system.

In relation to this matter, a web-based communication system has been developed to accelerate the reporting of HPAI H5N1 virus monitoring to decision makers at the National level. Communication methods between laboratories have been implemented within the **Influenza Virus Monitoring (IVM) Online** system, developed since 2011 and launched on 20th of May 2014.

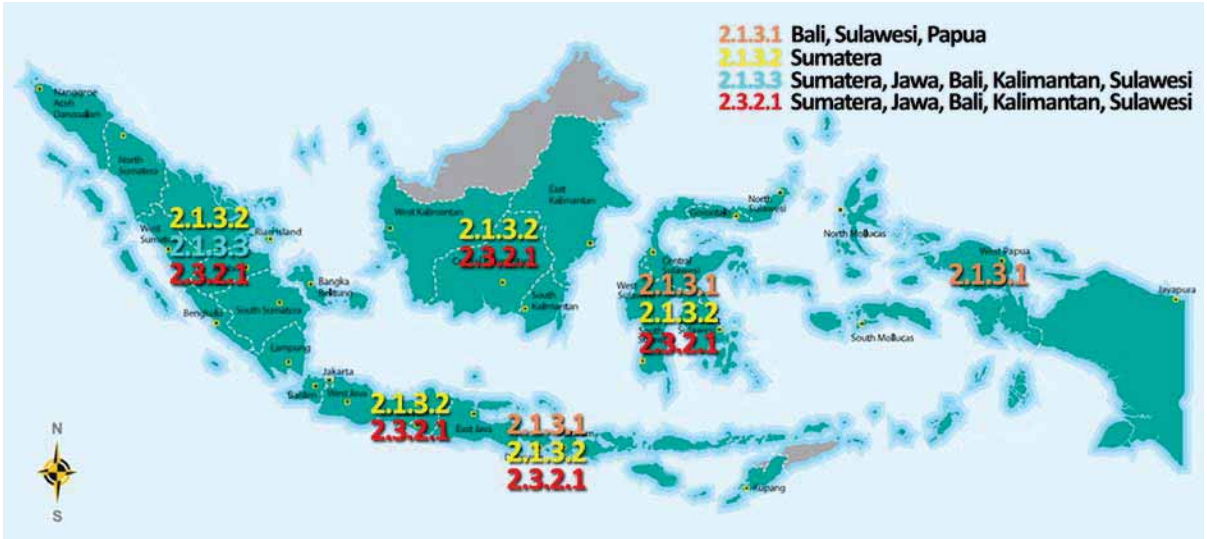
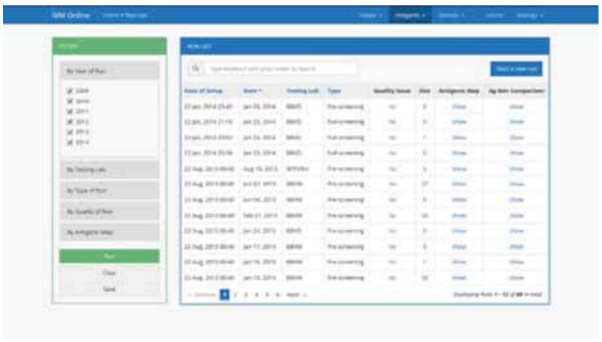


Figure 1

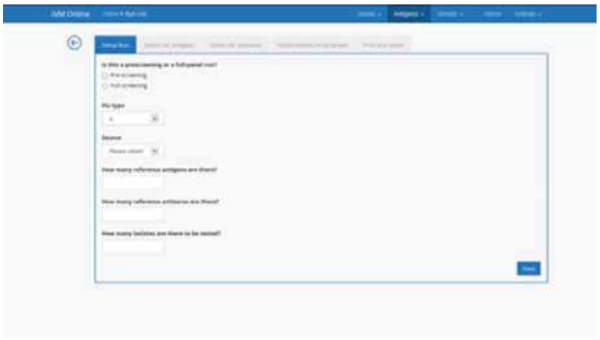
IVM Online is:
A web-based animal health laboratory network system in Indonesia that manages antigenic and genetic data of HPAI viruses circulating in Indonesia.

The implementation of **IVM Online** aims to:
1. Identify potential virus variants;
2. Identify and determine the strain for challenge virus, and
3. Monitor the efficacy of the vaccines used.

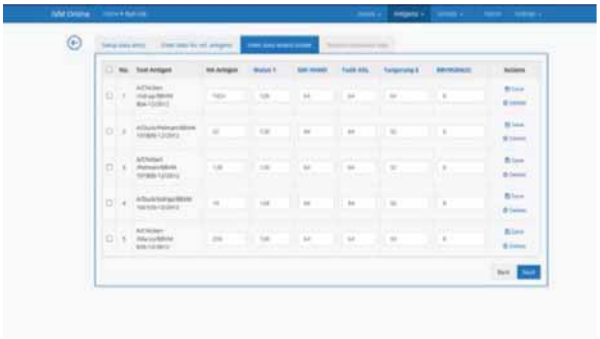
The main features of IVM Online
Within the **IVM Online** Software, there are many features that are useful to map out the HPAI virus situation in Indonesia. The main features are:
1. Antigenic Modules consisting of :
   a. Run list;



   b. Run set up;

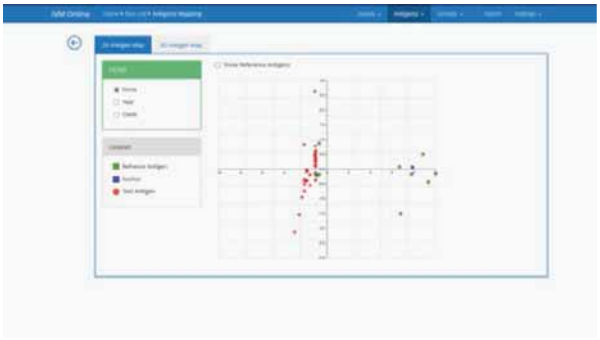


   c. Run data entry;

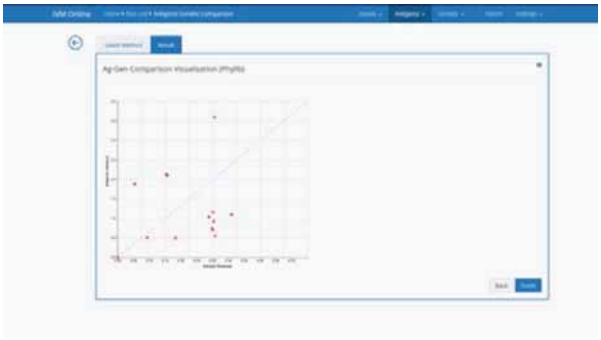


   d. Quality control summary report;

e. Antigenic mapping (displaying an antigenic map of a run);

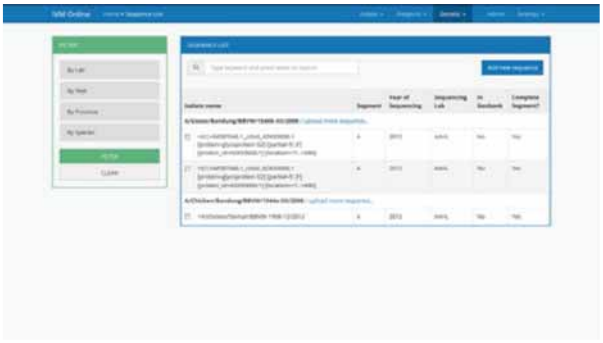


f. Antigenic and genetic comparison.

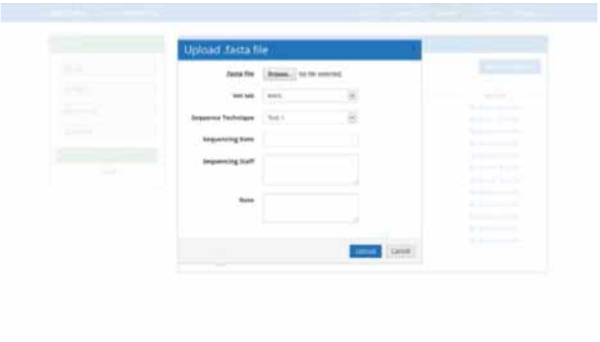


2. Genetic Modules consisting of:

a. List of sequences;

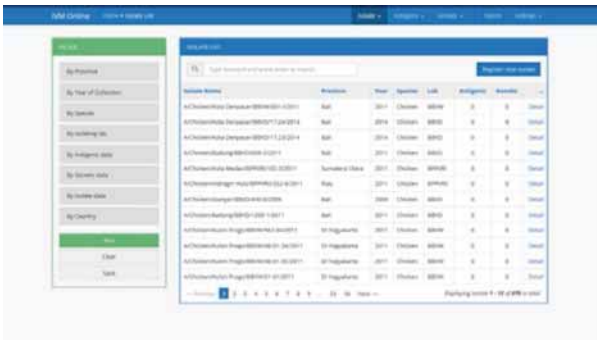


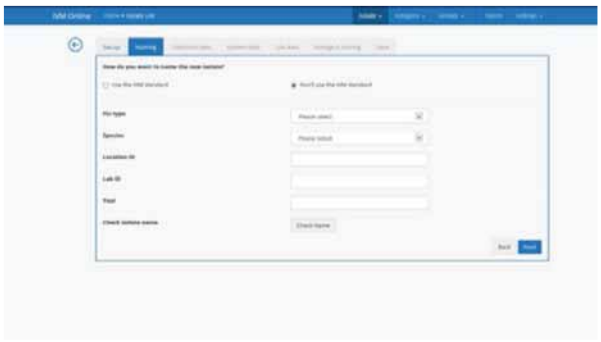
b. Sequence uploader;



c. Sequence viewer.

3. Isolate module

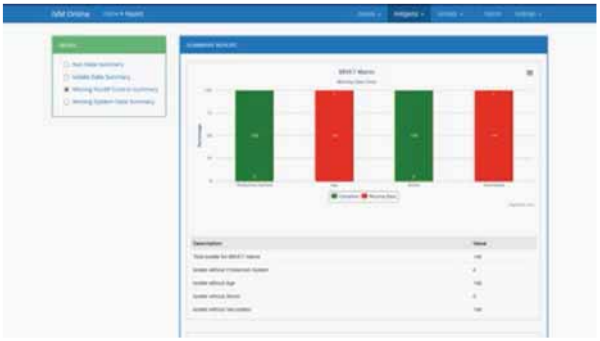




4. Reporting Module

a. Result conclusion ;

b. Report of a false result to guarantee data quality.

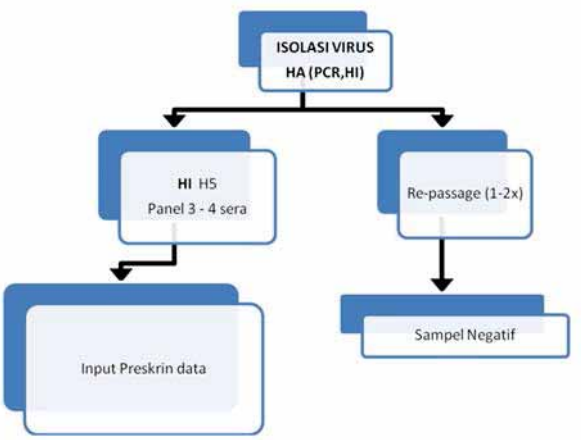


Members of IVM ONLINE

The members of the **IVM ONLINE** network are 8 diagnostic laboratories (DIC), BBPMSOH, PUSVETMA and BBLITVET. DIC Wates has been chosen as the focal point to control the activities of IVM Online.

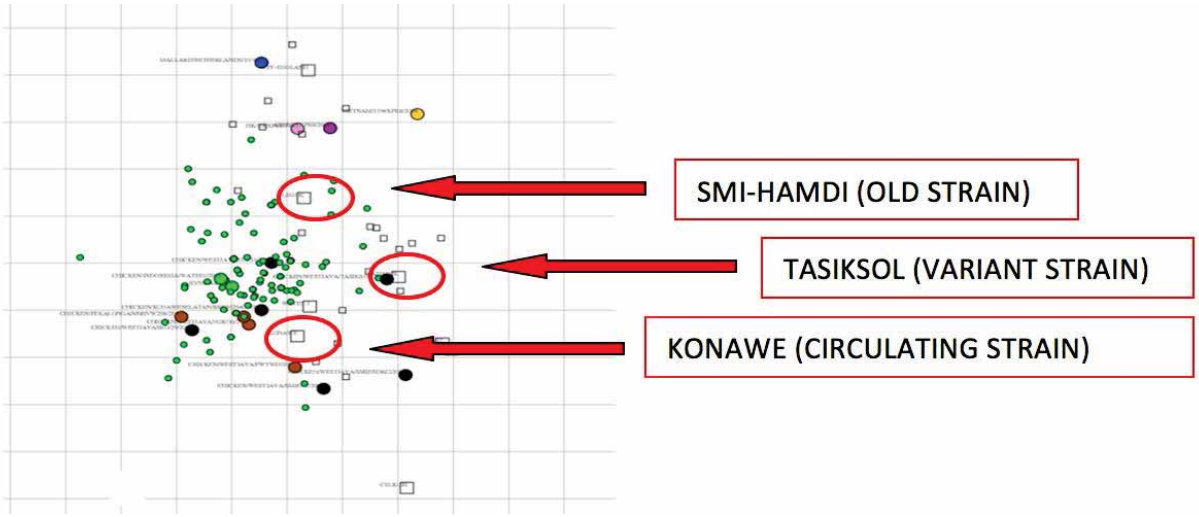
ACTIVITY PHASE

1. Members of **IVM ONLINE** routinely accept and process all AI samples from the field and only H5 viruses that have been through the **virus isolation process** will go to the next phase.
2. Those allantoic H5 HPAI viruses are identified by using hyper-immune sera like NDV and H5.
3. Positive H5 HPAI viruses are then **PRE-SCREENED** using the **PRE-SCREEN KIT** containing 3 antigens, 3-4 prime sera and H5 hyper-immune antiserum. The data of the HI titres along with epidemiological data of every isolate are then uploaded to **IVM ONLINE**.

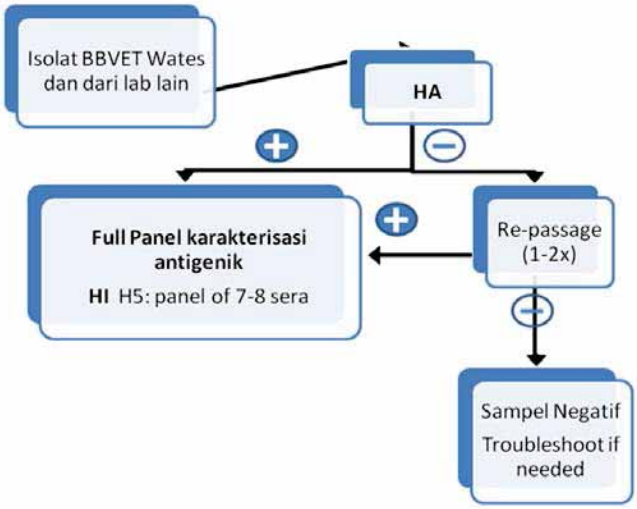


PRESCREEN PROCESS

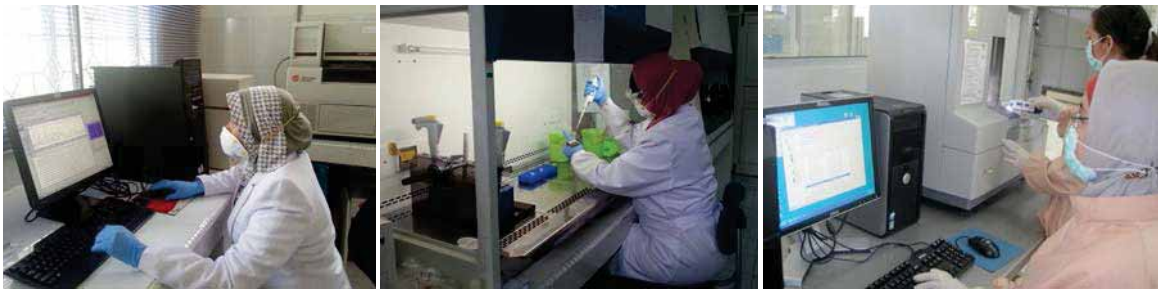
The reference antigens and antisera in each Pre-screen Kit used are always representative of viruses in circulation. The Pre-screen Kit constantly changes according to development of virus in the field. The image below is an example of Pre-screen Kit 1 used in 2010. To match the current situation in 2014, the Pre-screen Kit used is Pre-screen Kit 5, where the SMI-HAMDI strain has been removed and more antigenically relevant strains have been added, namely A/Chicken/West Java/Tangerang 6/2008, A/Chicken/West Java-Sbg/29/2007 and the A /VN 2.3.2.1 strain, which is genetically the same as clade 2.3.2.1 Indonesia H5N1 virus strains.



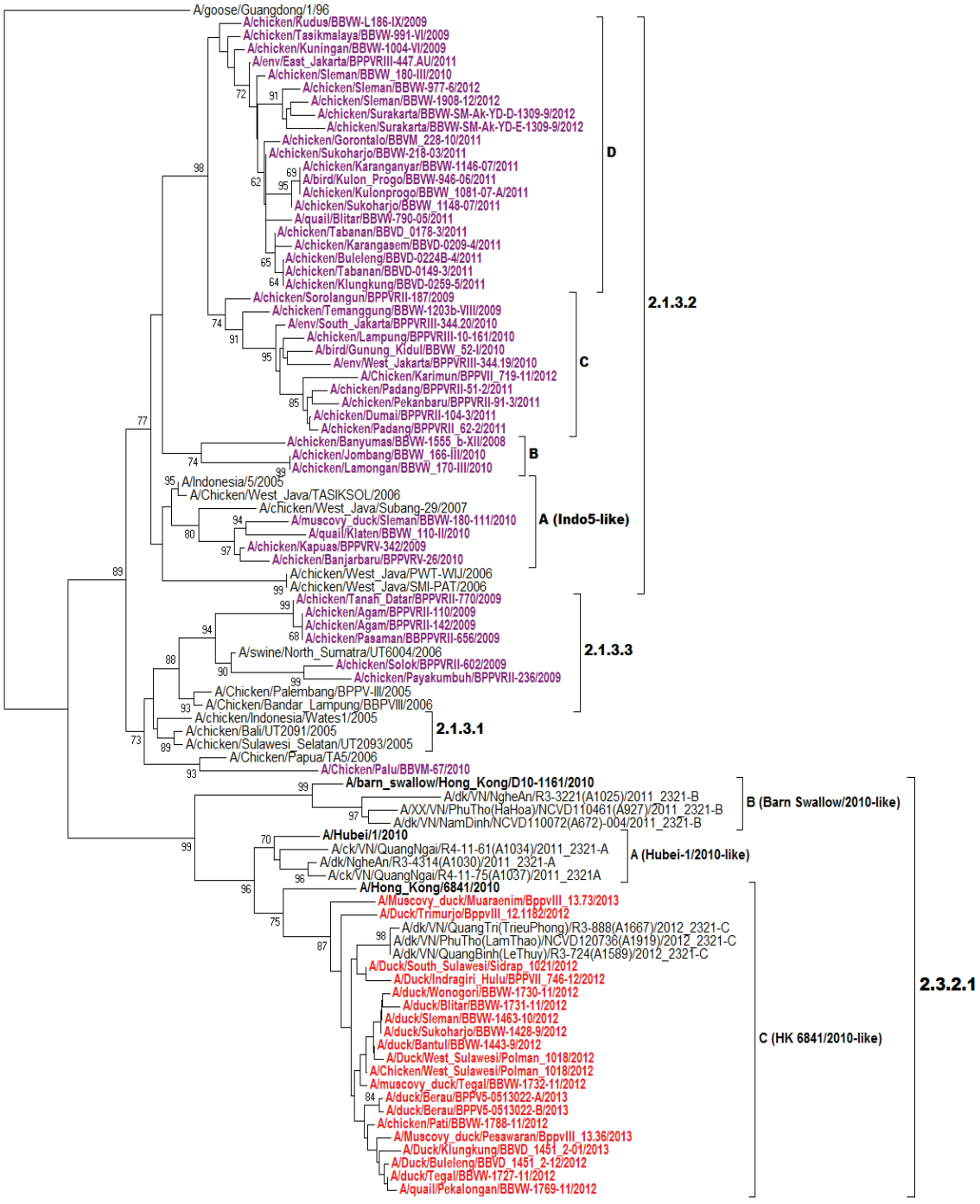
4. This pre-screen data is then analyzed by the **FOCAL POINT** (DIC WATES) by using an analysis module in IVM ONLINE to evaluate the selected isolate. All members have been requested to submit their selected isolates to DIC WATES to be propagated, and antigenically characterised using a pre-screen and a **FULL PANEL KIT** containing 7-8 antigens and antisera (prime and hyper-immune). The HI titres from the full panel antigenic characterization are uploaded to **IVM Online** and re-analyzed.



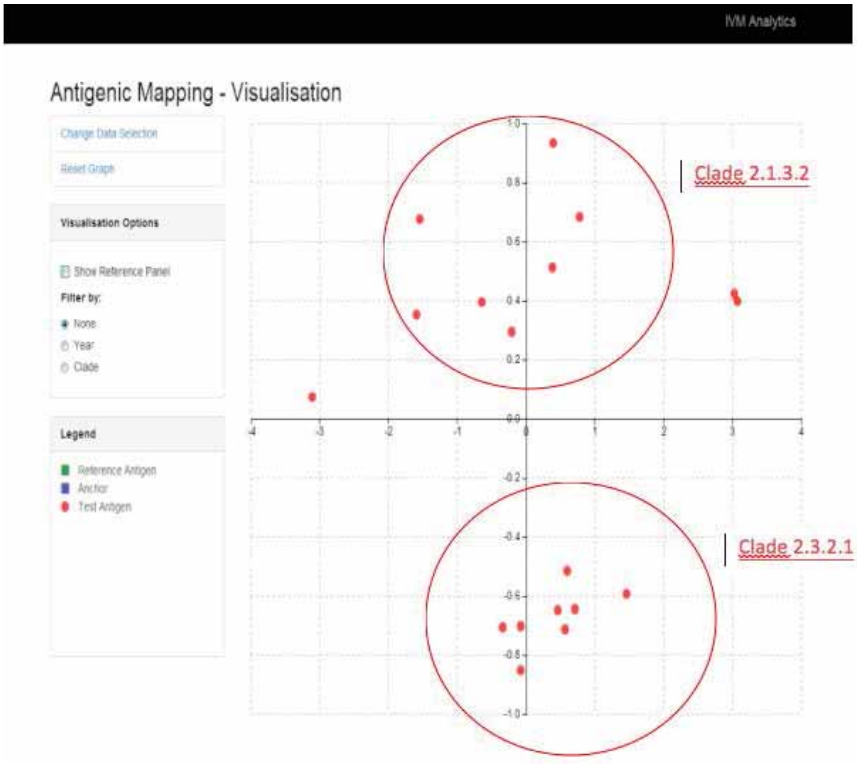
5. Identified variant strains are then sequenced by sequencing partners (DIC BUKITTINGGI, PUSVETMA, BBLITVET, and BBPMSOH). The results of the HA gene sequencing in the form of a **fasta file** are uploaded to **IVM ONLINE**.



6. Bioinformatics analysis of the sequencing and antigenic characterization results is conducted and mapped by the FOCAL POINT, and reported to the **Management Team**.



7. If the management team finds the presence of **ANTIGENIC SHIFT** in variant strains, then the management team reviews the data completely, including epidemiological and phylogenetic tree data of the variant strain; if necessary, the management team will immediately **recommend** changing the challenge virus strain to the Director of Animal Health.



8. All of the activity flow above can be monitored by the Directorate General of Livestock and Animal Health Services online.

RESULTS

1. **IVM ONLINE** is a system to select challenge and vaccine seed strains objectively.
2. Faster antigenic and genetic characterization which allows decision making for production of AI vaccine against the new clade (Afluvet) that matches the type of HPAI in circulation.
3. Established pre-screening and full panel screening methods using HI protocols with standard panel reagents for antigenic characterization.
4. Established a web-based database **(IVM ONLINE)** which contains bioinformatics modules for antigenic and genetic characterization, as well as visualization.
5. Update the molecular diagnostic method (PCR) for Type A, H5N1 clade 2.1.3 and 2.3.2.

IMPACT

1. Increases the capabilities of animal health laboratories in Indonesia in performing antigenic and genetic characterization of influenza virus.
2. Improves knowledge about circulating AI viruses and informs vaccination policies.
3. **IVM ONLINE** can provide recommendations for determination of a candidate challenge strain or vaccine seed strain.

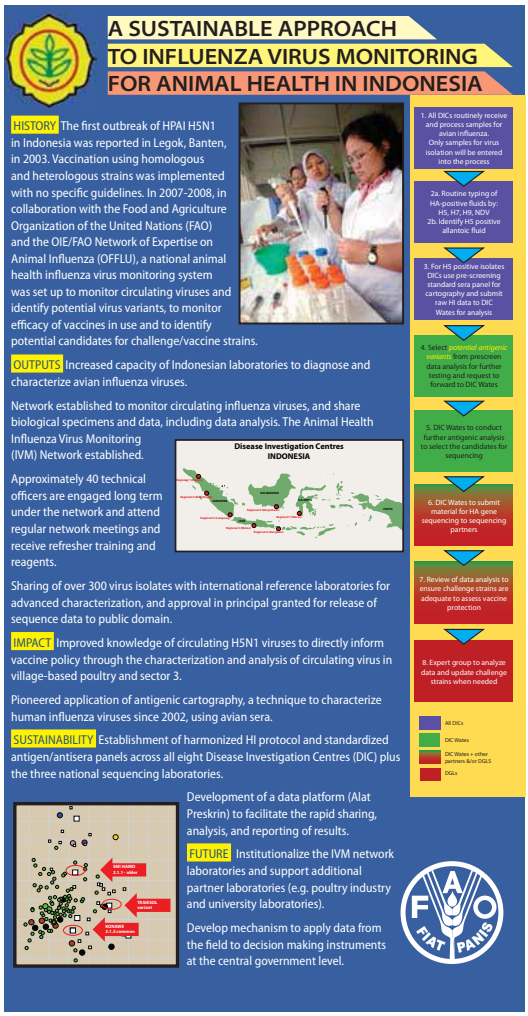
QUALITY ASSURANCE AND SUSTAINABILITY OF IVM ONLINE

a. **IVM ONLINE** activities conducted by competent officers/staff.
b. **IVM ONLINE** staff/officers competence is developed through **Competency Based Training**.
c. Training is determined and performed both internally by focal points and externally.
d. For **QUALITY ASSURANCE** and **QUALITY CONTROL**, the implementation of **IVM ONLINE** activity follows **TECHNICAL GUIDELINES** and **STANDARD OPERATIONAL PROCEDURES – SOP**.
e. Meetings between **IVM ONLINE** members are held every year to ensure the continuation of the **IVM ONLINE** system.

FUTURE PLANS

- Expanding the bioinformatics module within the **IVM Online** system to detect other HPAI viruses such as H7N9, H7N7 etc, related to epidemiological data.
- Developing a linkage mechanism between **IVM Online** and **iSIKHNAS.**
- Expanding the membership of **IVM Online** to include laboratories from universities and the private sector.
- Strengthening the regional and global laboratory network to face the emergence of many different zoonotic pathogens.
- Forming an Influenza Virus Monitoring (IVM) Team determined by the Director General of Livestock and Animal Health Services to ensure the continuation of **IVM Online**.

PUBLICATION



POSTER

Surveilans pada tingkat molekular: Mengembangkan jaringan yang terintegrasi untuk mendeteksi varian virus flu burung di Indonesia.

Hendra Wibawa[1], Pudjiatmoko[2], Fadjar Sumping Tjatur Rasa[1], Sri Handayani Irianingsih[3], Rama Dharmawan[1], Muhammad Azhar[1], Elly Sawitri Siregar[1], Nining Hartaningsih[1], James McGrane[2], Frank Wong[2], Paul Selleck[2], John Allen[2], Ivano Bisor[2], Mia Kim Torchetti[4], Gwenaelle Dauphin[5], Filip Claes[5], Wiryadi Sastraningrat[1] dan Peter Daniels[2]

[1]Direktorat Kesehatan Hewan, BBVET Wates, Yogyakarta. [2]Direktorat Kesehatan Hewan, Direktorat Perternakan dan Kesehatan Hewan, Jakarta. [3]Unit Respon Cepat, Direktorat Perternakan dan Kesehatan Hewan Jakarta. [4]FAO Indonesia, Jakarta. [5]AAHL, Geelong, Australia. [6]FAO OFFLU, Rome, Italy. [7]EuRenote, Universitas Udayana, Bali. # menunjukan afiliasi penulis.
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Kata Kunci: kartografi antigenik, flu burung, bioinformatika, H5N1 HPAI, jaringan laboratorium, OFFLU

Abstrak

Sejak 2006, Indonesia telah menggunakan vaksinasi sebagai metoda untuk mengendalikan H5N1 HPAI. Dalam kurun waktu tersebut, virus telah mengalami perubahan antigenik secara bertahap, yang memunculkan diversifikasinya perubahan seed strain untuk vaksin dan antigen untuk diagnostik. Untuk meningkatkan sistem monitoring evolusi virus, Pemerintah Indonesia, dengan dukungan dari FAO/OFFLU, telah mengembangkan jaringan nasional dimana isolat H5N1 dikarakterisasi secara antigenik dan genetik. Jaringan surveilans molecular ("Influenza Virus Monitoring" atau Monitoring virus Flu Burung atau disingkat "IVM") didasarkan pada jaringan regional laboratorium kesehatan hewan diagnostik, yang didukung dengan sistem pengolahan data berbasis web ("IVM Online"). Jaringan IVM Indonesia dapat menjadi referens untuk negara lain yang ingin mengembangkan jaringan laboratorium untuk surveilans flu burung dan patogen lainnya.

Pendahuluan

Sub tipe H5N1 flu burung dengan patogenitas tinggi (HPAI) menyebar ke Indonesia di 2003, dan pemerintah menjawab kebijakan dengan menggunakan penggunaan vaksinasi untuk pencegahan penyakit pada tahun 2004 [1]. Indonesia adalah satu diantara empat negara, selain Cina, Mesir dan Vietnam, yang secara konsisten menggunakan vaksinasi sebagai strategi pengendalian H5N1 dalam 10 tahun terakhir [2].

Seperti telah diketahui bahwa virus H5N1 mengalami evolusi yang cepat dan konsisten, melalui proses perubahan genetik (antigenic drift) dan pencampuran (reassortment). Masalah spesifik yang dihadapi oleh otoritas veteriner adalah mengetahui kapan diperlukan tindakan ketika variasi atau adanya clade baru telah terdeteksi. Pada prakteknya hal ini akan berakibat pada dibutuhnya perubahan rekomendasi penggunaan galur vaksin dan atau perubahan isi vaksin dari vaksin yang berisi satu antigen ke vaksin yang memiliki 2 atau lebih antigen. Selain itu, protokol diagnostik kemungkinan juga akan mengalami perubahan.

Dalam sistem surveillans hal ini dapat diartikan sebagai masalah sulit dalam pengambilan keputusan. Contoh: Misalnya upaya monitoring virus mampu mendeteksi perubahan, pertanyaannya, apa yang menjadi patokan untuk melakukan tindakan untuk merespon hasil deteksi? Pertanyaan ini hanya dapat dijawab secara efektif apabila otoritas veteriner memiliki data berkualitas yang tersedia secara tepadu dan tepat waktu.

Dalam paper ini dijelaskan suksesnya penerapan sistem monitoring HPAI yang terkoordinasi dan terpadu berdasarkan jaringan laboratorium kesehatan hewan di Indonesia (Gambar 1). Termasuk juga pengembangan protokol yang standar untuk proses karakterisasi antigenik dan sistem pengolahan data berbasis web, yang disebut "IVM Online".

Gambar 1. Anggota jaringan laboratorium dengan penjelasan peran utama masing2 anggota dalam jaringan monitoring.

Materi dan Metode

Pengembangan Sistem Monitoring Virus Influenza (IVM) dimulai pada akhir 2007 dibawah proyek FAO dan Direktorat Jendral Peternakan dan Kesehatan Hewan (DGLAHS) Kementrian Pertanian Indonesia. Kegiatan ini mulai di implementasikan di Indonesia dengan bantuan OFFLU (http://www.offlu.net/), yang merupakan jaringan para ahli influenza hewan dari FAO dan OIE [3]. Para ahli dari proyek ini berasal dari Australian Animal Health Laboratory (AAHL) atau Laboratorium Kesehatan Hewan Australia, Southeast Poultry Research Laboratory (SEPRL) atau Laboratorium Penelitian Unggas Asia Tenggara dan

PAPER