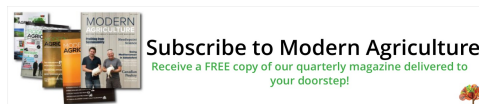


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P O U R E S

Is a Rapid Detection Tool for Bird Flu on the Horizon?



From China to the United States, the avian influenza continues to endanger both poultry and man. Also known as bird flu, this viral disease has rendered 3 to 5 million people severely ill while 250,000 to 500,000 have lost their lives. Type A, especially strains A (H5N1) and A (H7N9), has proven to be deadlier, putting governments and farmers alike under pressure to ensure the health of birds and ultimately the people who consume them.

According to health experts, the lack of information on this disease is what causes fear among farmers and consumers. Moreover, while the FDA had approved a H5N1 vaccine in 2013, it was limited to the National Stockpile and left unavailable for commercial use. Besides, the Centre for Disease Control (CDC) reported that the vaccine was ineffective as it required multiple dosages. Now, new vaccines are underway, but none of them has been approved so far. This is why preventing the spread of the infection is the best way to keep the disease under control. And prevention in this case starts with effective surveillance

Current methods for detecting avian flu

The first and most popular method for detecting avian influenza A (H7N9) is reverse-transcription polymerase chain reaction (RT-PCR). Developed by the World Health Organization Collaborating Center in Beijing, the protocol is designed to detect infectious agents such as the avian flu virus. What made RT-PCR stand out was the fact that it took only four hours to diagnose birds, which is a much shorter time than the 21 days traditionally used by the Paris-based World Organization for Animal Health (OIE). As a result, it is less expensive than its counterparts. Unfortunately, since the method is not designed for commercial use, it requires expensive equipment which may not be available for the people who actually need it.

Another popular method is enzyme-linked immunosorbent assay (ELISA), which determines the concentration of analyte (e.g. antibodies or antigens) in solution. By analyzing the colored end product, the amount of analyte can be determined and ultimately how far the disease has progressed. Now, ELISA is one of the most sensitive immunoassays available. However, it still lacks the sensitivity and specificity required for quickly curbing an avian flu epidemic. Even though effective commercialized immune assay kits are available to help farmers, these do not provide an accurate number indicating analyte concentration. As a result, they have no other option than to destroy the whole coop at times.

A third common detection tool is nucleic acid sequence-based amplification (NASBA). It can identify the disease through its antigens' RNA using cost-effective ELISA plate readers. Used heavily in China and Japan to monitor H5N1 avian influenza in poultry, NASBA can also be standardized across different laboratories easily. While scientists have thought of developing point-of-test devices based on this technique, its limitations have made them re-assess their decision. First off, as in the case of most RNA amplification procedures like RT-PCR, the integrity of RNA is a cause of concern. In addition, farmers themselves will not be able to carry out this diagnosis since the target sequence should be between 120 and 250 nucleotides or else they will be amplified less efficiently.

To sum everything up, most of the commonly used techniques for detecting avian influenza are inaccurate, expensive and unavailable for those directly in contact with the infected animals. This explains why chances of re-infection are high while the opportunities to recover financial losses afterwards are quite low. However, based on the findings in a paper by Longyan Chen and Suresh Neethirajan from the BioNano Laboratory in University of Guelph's School of Engineering, a quicker detection tool may be on the way.

Proposed new method

In their paper "A Homogenous Fluorescence Quenching Based Assay for Specific and Sensitive Detection of Influenza virus A Hemagglutinin Antigen", Chen and Neethirajan propose the use of the powerful and sensitive fluorescence resonance energy transfer (FRET) tool along with a homogenous fluorescence-quenching assay for optimum results.

FRET is a powerful technique and one of the few tools which can measure the smallest changes in the interaction of molecules or atoms. Studying it, different groups noticed its ability to detect and separate the nucleic acid sequences of the virus. However, the assays (tests to determine the components of a certain subject) traditionally used for FRET required more processing with the help of additional reverse transcription PCR. Another issue is that antibody-based assays were never investigated before due to the large size of antibodies, which block the interaction between molecules and reduce the efficiency of the diagnosis.

However, Chen and Neethirajan propose improving the results of FRET through a homogenous fluorescence-quenching assay. Uniform in structure and composition, this assay consists of glycan conjugated quantum dots

(Gly-QDs) and an antibody-modified gold nanoparticles (Ab-Au NPs) pair. The first is a fluorescent agent, which means that it emits light, while the second is a fluorescence quencher, i.e. agent that reduces the substance's ability to emit light. On their own, Gly-QDs and Ab-Au NPs do not react. However, in the presence of antigens such as avian flu, Ab-Au NPs robs QDs of their florescence.

Testing the reaction of both components, the scientists uncovered that high sensitivity is possible for both H1N1-HA and H5N1-HA, and thereby can differentiate whether the flu strain of human patient is due to avian influenza or not. Regardless, considering the fact that this is the first type of experiment that used non-nucleic acid conjugated nanoparticles in a FRET analysis, further research is bound to follow. Moreover, Chen and Neethirajan may get to see the biosensors they proposed for quickly detecting the disease and taking preventative measures. How effective is the method—really?

The 2015 hypothesis that Gly-QDs and Ab-Au-NPs can enhance the functionality of FRET analysis can be supported by a 2012 theory proposed by Cheng-Chung Chou and Yi-Han Huang from the National Research Council of China (Taiwan). When integrated with a homemade optical sensor made from a UV LED, mini CCD spectrophotometer and an ITO glass slide, the assay system could be used for nucleic acid analysis and on-site surveillance.

Practically speaking, the proposed Gly-QDs and Ab-Au-NPs assay is quite convenient. Since it does not require additional washing steps, it can save sensing time. This feature alone can ensure that farmers automate the detection procedure, providing farmers and the commercial industry with a device they can use conveniently on their own without resorting to the services of professional labs early on.

Considering the threat which avian influenza poses to human health and the growth of the agricultural sector, investing in disease control strategies is vital. Therefore, theories should be thoroughly tested out to equip farmers with tools that can efficiently detect the virus before it wreaks havoc on their main produce. Luckily, governments are funding studies ranging from cell culture of the viruses to vaccines and all the way to changing wild bird migration patterns to keep them at bay. Through their efforts so far, H5N1 is relatively contained and its speed and mutation are limited. However, until a proper monitoring solution is implemented, it will be a while before the death toll can be minimized and the next flu pandemic avoided.

words: Dr. Suresh Neethirajan

Tags: *avian flu , bird flu , detection , disease , influenza , Poultry*

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