IAEA tackles Bird Flu diagnosis

The First Research Coordination Meeting (RCM) for the Coordinated Research Project (CRP) on “The early and rapid diagnosis of transboundary animal diseases such as avian influenza” was held at the IAEA, Vienna, 19 to 23 March 2007. The RCM brought together Research Contract and Agreement holders as well as observers. The meeting reviewed the work of the participants through presentations and discussions and specific work plans were discussed. Observers illustrated new technologies for exploitation in rapid diagnosis and one in particular was the focus of great interest, offering the potential to perform all sample analysis and reporting in a single unit. The announcement brought great press interest.

Technologies

Current technologies range from penside or dipstick technologies to multiple biomarker detection systems like microarrays. The selection of diagnostic tools should be based on the most effective, appropriate, affordable, and maintainable platforms available for the desired purpose. The tools should reflect the diagnostic urgency to promote timely and informed decision making.

The use of multiple platforms (in sequence or in parallel) should increase confidence in the diagnostic results, aide in risk-assessment, and to ensure that appropriate response is taken. Penside or dipstick technologies exist and are beneficial, but development and validation of new tests require the development of biological reagents and commercial partnership for sustainable production and quality assurance.

The real-time PCR is currently the device of choice for genetic amplification, however, it is only one of several methods available and not appropriate in all environments or situations. Other amplification technologies should be evaluated because the cost of current amplification technologies hinders the sustainability of this technology.

Biosensors, multi-bead flow-through, and other emerging technologies are coming available and efforts should be made to encourage the development of this technology for agricultural purposes.

Remote sensing devices that would allow the early forecasting or detection of disease should be investigated. This would include satellite monitoring of environmental factors associated with potential disease outbreak conditions, information search tools to monitor open source internet-based material, and the capability to track and monitor the health of an individual or sentinel or animals from a remote location.

Integration of currently available databases that can filter collected data sets in an electronic matter (not requiring human resource capital) is needed. It is recognized that understanding baseline data and its relevant analysis for disease forecasting is required.

Avian Influenza threat

The rapid spread of avian influenza (AI) between countries and into new species, continues to intensify the risk of a pandemic, and critically emphasizes the need for global efforts to provide early detection and diagnosis of high-threat transboundary animal diseases. The findings and direction proposed during a previous Joint FAO/IAEA Consultant’s Meeting were used by the international community in introducing new technologies for disease detection and diagnosis to the member states.

Additionally, the needs for enhanced collaborative efforts involving private industry, governmental agencies, and researchers were recognized. The progress in this area exceeded earlier expectations in advancing appropriate technologies, including robotics, for field and laboratory use. Continued efforts are needed to improve affordability; enhance flexibility and to provide seamless integration from sample collection through to reporting that would allow
Application of Nuclear and Related Techniques to the Diagnosis of Avian Influenza

Highly pathogenic avian influenza (HPAI), now commonly known as “bird flu”, is caused by the infection with some strains of Influenza A virus. The different strains of this virus are classified into subtypes on the basis of their two external proteins named haemagglutinin (H) and neuraminidase (N). Techniques that are implemented for the diagnosis of avian influenza aim at demonstrating first the presence of the causal virus in pathological samples and then at assessing it’s pathogenicity. Indeed, only some strains of avian influenza, highly pathogenic (HPAI), are at the origin of outbreaks and: they belong to the H5 or H7 subtypes. The current avian influenza outbreak which started in Asia in 2004 is caused by a virus of H5 subtype. In addition, this virus was further characterized as of the N1 subtype which is able to cause deaths in humans.

Usually, from a pathological sample, virus is first isolated after growth in embryonated fowl eggs, which takes 4-7 days to complete. The subtype of the isolated virus is then identified by a battery of specific antibodies produced against the different H (H1 to H15) and N (N1 to N9) proteins. This method of identification can only be made in specialized laboratories. To confirm a subtype’s pathogenicity, the isolate is then inoculated into 4-8 week-old susceptible chickens. For the World Organisation for Animal Health (OIE), strains are considered to be highly pathogenic if they cause more than 75% mortality in inoculated chickens within 10 days.

An alternative way to demonstrate the presence and characterize the influenza virus in the pathological samples, is the specific detection of RNA by nucleic acid amplification techniques (PCR and PCR sequencing, using either fluorescent or isotopic [P\textsuperscript{32}, P\textsuperscript{33} or S\textsuperscript{35}] markers). This molecular approach takes 1-2 days to complete. Furthermore, it is foreseen that this technology could be applied to provide early warning tools.

The RCM

Research contract holders gave country reports highlighting what they were doing concerning the control of AI. Power Point presentations were shown in individual files. The expert as well as observers and commercial companies also participated in discussions and most made presentations, most of which were shown as Power Point in individual files.

Discussions ranged from defensive measures being taken by countries as yet unaffected; to countries where there was active AI infection. Surveillance was discussed whether purposive or passive. There was believed to be a need to re-examine the criteria behind both measures in terms of statistical design. The problems of public awareness were illustrated whereby campaigns to educate the public resulted in swarming of laboratories with wild birds killed by any means, road kills etc., and the capacity for testing was questioned in some laboratories. The specificity of tests to discriminate between AI isolates was discussed down to the highly pathogenic level. The use of dip stick technologies was discussed.

Emphasis was placed on fitness for purpose of any systems, including those being currently used and it was felt that there was poor validation data for most tests used and on the market. The use of devices in the field and the potential to instantly both perform a test and report it was discussed. The feasibility of this has been greatly increased through the development of machines able to extract and process a sample through a thermocycling PCR as a single operation. Of most interest was the devise shown by DxNA Company. The use of LAMP PCR was also thought to be important to offer flexibility to detection systems.
Conclusions

1. The DxNA machine as applied to testing for AI should be further investigated.

2. The specific primer sets to allow identification of AI as well as discrimination from Newcastle Disease Virus (NDV) in the DxNA machine were agreed.

3. It was agreed that the DxNA Company would produce defined modules (cassette for machine with specific primers for AI.

4. It was agreed that the modules and machines would be provided to Sweden, USA and Australia so that they could rapidly verify the efficacy of the primers as well as test the machine-product claims.

5. It was agreed that this process should be finalized by the end of August, 2007.

6. It was hoped that Research contact holders could receive machines and modules as soon after August 2007 as possible, to obtain validation data comparing the performance of the DxDNA machine and modules with stored and fresh samples as well as taking the machine to the field. The methods to be used will be discussed with Technical officers before active work begins on this.

7. It was stressed that the ability of the machine to send data directly to a central collation point would be an enormous advantage in certain scenarios for rapid response to diseases. It was agreed that the necessary adaption could be made easily but that some discussion as to the exact nature and various forms of the communication peculiar to different national was needed.

8. The LAMP system for AI diagnosis will be developed and primers selected. A machine is to be tested and further developed through the IAEA, that can read the reaction (turbidometric) of the PCR reaction in the LAMPCR and also transmit data in real time. The Netherlands laboratory agreed to make the necessary adaption.

The meeting brought together a diverse range of interested parties as well as eliciting great media interest. A considerable amount of cross fertilization of technologies was made through contacts which may well lead to improve the end users ability to diagnose disease. One golden aim; that of being able to process and test a sample in a single process as well as transmit the result in real time, breaks down in the often painfully slow process between the field, the laboratory and action.

The CRP will be taking a leading role in justifying the claims of the manufacturers of the machine as well as developing the process for disease reporting. It is hoped and expected that the systems deployed will work and that within the next year a fully functional direct test and data transmission system will be in place for AI. Such systems are adaptable to any livestock disease for which diagnosis can be based on detecting a specific nucleic acid, so that they should be of great appeal to countries with the need for constant surveillance against foreign diseases where a rapid response is needed. The biggest drawback in identifying disease and action is the reporting of an incident and sending of samples for confirmatory laboratory work.