Early Warning

by Sándor Belák

Avian flu has spread to 51 countries—36 this year alone—many of which are densely populated and deprived.

Can nuclear technologies help detect such contagious diseases?

Highly contagious animal diseases are transboundary threats of growing concern.

Such diseases include foot-and-mouth disease, swine fever, rinderpest—and the highly pathogenic avian influenza or "bird flu" making so many headlines. The diseases—which experts call TADs, (transboundary animal diseases)—are regularly emerging and re-emerging all around the world. They cause billions of dollars in losses and threaten the health, lives and livelihoods of millions of poor farming families and their neighbours.

Within the last 18 months alone, the Office International des Epizooties (OIE, World Organisation for Animal Health) reported a high number of TAD outbreaks on several continents—foot-and-mouth disease in Africa, Asia and South America, classical swine fever in Africa, Asia and Europe and rinderpest in Africa and Asia. And, most recently, there has been intense media coverage of the highly pathogenic avian influenza (HSNI) that has caused severe outbreaks in Asia, Africa and Europe. Many birds, animals, and people became sick, and millions died.

The costs of TAD outbreaks should be viewed both in terms of the efforts to bring the disease under control and the consequent loss of livelihoods. As an example, with regard to the UK foot-and-mouth disease outbreak in 2001, the cost to the public sector was estimated at over 4.5 billion euro, and the cost to the private sector at over 7.5 billion euro. The ethical problem raised under the eradication strategy and the social consequences of the slaughter of large numbers of animals are just some of the hidden costs to consider when evaluating the effects of these threatening diseases.

Today more organizations and experts are banding together to prevent and combat TADs. They include veterinary health services, research institutes and international organizations, including the IAEA and the Food and Agriculture Organization, which run a joint division in Vienna, Austria. The joint FAO/IAEA programme is working on the rapid detection of emerging diseases, including bird flu, and using nuclear and radiation techniques in the process.

The problems are serious and challenging, but nuclear technologies may offer a solution.

For most developing countries, TAD detection is still vital. The bottleneck is their inability to rapidly detect the virus and to determine early enough whether it is H5N1 or another subtype, so that authorities can take appropriate control measures. Serious efforts are focused on the early detection of the agents. Timely recognition of such viral infections would prevent the spread of the diseases to large animal populations in huge geographic areas. Thus, the development of novel, powerful diagnostic nuclear and nuclear-related assays is a crucial issue today in veterinary research and animal health care.

Molecular virology offers a range of new methods, which are able to accelerate and improve the diagnosis of infectious diseases in animals and in man. The molecular detection assays, like the polymerase chain reaction (PCR) technologies, provide possibilities for a very rapid diagnosis. The detection of viruses can be completed within hours or hopefully even within minutes with a sensitivity level of less than one pathogenic organism.

Molecular approaches have contributed significantly to the rapid detection of well-established, as well as newly emerging, infectious agents such as Nipah and Hendra viruses or corona viruses in the SARS scenario and the detection and molecular characterisation of the highly pathogenic avian influenza H5N1 subtype that threatens the world today. The nucleic acid amplification assays, although they were at first expensive and cumbersome, have become relatively cheap and user-friendly tools in the diagnostic laboratories.

In Sweden, the first diagnostic PCR assays were established as early as 1987, just two years after the first description of the PCR principle. In the last two decades more than 50 PCR assays were developed and validated here, and are in routine use in the diagnostic laboratory.

When examining the genetic relatedness of various viruses, the purpose is not to achieve wide-range detection, but to

obtain a high phylogenetic resolution or fingerprint of the particular virus or isolate. For this, the variable genomic regions of the viruses are targeted and these give a direction of virus evolution often indicating the origin of the original infection. Such phylogenetic PCR assays are used to group pestiviruses, including classical swine fever virus and bovine viral diarrhoea virus and to classify pathogenic isolates (H5N1 as a case in point).

The PCR assays of high phylogenetic resolution are useful tools for the rapid identification of various virus variants. The genetic identification is very exact and rapid (several days or hours). The spread of virus variants can be traced and cut rapidly, in order to prevent distribution of the virus to large geographic areas.

The rapid phylogenetic identification and tracing of the viruses is termed "molecular epizootiology." For example, such studies were conducted when genetic variants of classical swine fever virus were identified in several countries of Central Europe and when it was hypothesised that EU and US genotypes of the porcine respiratory and reproductive syndrome virus evolved from a common ancestor found in East Europe.

The real-time PCR assays provide novel rapid means of virus detection. The diagnostic work can further be automated by using robotics for nucleic acid extraction and pipetting. Compared to previous amplification assays, the real-time PCR has a further advantage: it allows running *quantitative PCR*, allowing an estimation of viral load (the amount of virus in the blood). The quantitative aspect is crucial when a virus commonly found in animals is possibly causing symptoms in relation to viral load, for example feline coronaviruses or porcine circovirus 2. The measurement of viral load is also important when estimating the effects of anti-viral treatments, especially in human virology.

To assure the reliability of the diagnostic PCR assays, it is important to incorporate internal controls. By including such an *intrinsic control* with its specific reporter fluorophore, we obtain information on the sample quality and on pipetting errors. Simultaneously, the system shows the amplification of the target nucleotide sequences and provides safety for the diagnosis.

Today, both national and international authorities require rigorous proof that the diagnostic assays are as reliable as possible. International agencies like the OIE, FAO/IAEA, national research institutions and commercial companies make great efforts to agree on international standardisation.

Considering these requirements, diagnostic laboratories have started the validation and standardisation of the routine diagnostic PCR assays. For example, the EN ISO/IEC 17025:2000 standard gives directives for an accredited laboratory and it specifies many important parameters. OIE also has published, in 2000, a standard for the validation of diagnostic assays in the veterinary field.

How fast can we identify and characterize a pathogenic virus like bird flu?

Using molecular approaches, the time is one or two days — much faster that conventional methods. In Sweden, a one-step, real-time PCR assay has been developed for the rapid and simultaneous detection of a broad spectrum of influenza viruses, including those associated with highly pathogenic avian influenza.

Rapid identification and detection can serve as an early warning tool, an important need particularly for developing countries. The simultaneous detection of different sub-types of avian influenza allows authorities to monitor the occurrence of influenza strains in wild birds, in farmed poultry, and in mammalian species. The method provides a very rapid and highly reliable molecular tool for diagnosing one of the world's worst transboundary animal diseases.

Sándor Belák works in the Joint Research and Development Division, Department of Virology, Swedish University of Agricultural Sciences (SLU) and the National Veterinary Institute (SVA), in Uppsala, Sweden.
E-mail:sandor.belak@sva.se.

Bird Flu Background

Technically, avian influenza or "bird flu" is known by numbers and letters—HPAI of the sub-type H5N1.

Today's avian flu outbreak started in Asia in 2004 and is caused by a virus of the H5 sub-type. Additionally the virus was characterized as of the N1 sub-type—an important finding which revealed that the flu could be deadly to humans.

HPAI is caused by the animal's infection with some strains of influenza-A virus. The strains are classified into sub-types on the basis of their two external proteins, named haemagglutinin (H) and neuraminidase (N).

How is the virus identified and detected? Usually, from the pathological sample, the virus is first isolated in the embryos of chicken eggs. This takes between four and seven days. The sub-type of the isolated virus must then be identified by a a battery of specific antibodies raised against the different H and N proteins.

Identification can only be made in specialized laboratories. To confirm a sub-type's pathogenicity, the isolated virus (isolate) has to be subsequently inoculated into chickens that are four to eight weeks old and susceptible to the virus. Strains are considered to be highly pathogenic if they cause more than 75% mortality in inoculated chickens within ten days.

A big problem is that existing detection procedures are time-consuming. Fortunately, faster methods are emerging, with the support of the IAEA, FAO and other institutes and organizations.