

WVPA Asia Meeting a great success



In September the World Veterinary Poultry Association (WVPA) held its 2nd WVPA Asia Meeting in Bangkok, Thailand and had Testing & Monitoring as its theme.

In the opening paper, Roberto Soares from Ceva considered key aspects when monitoring flocks vaccinated against Newcastle disease with rHVT-F vaccine.

He concluded that recombinant rHVT-F vaccine expressing the F protein applied in the hatchery induces homogenous and solid immunity against Marek's disease and Newcastle disease by stimulating cellular and humoral immune responses.

However, many external factors may interfere with the efficacy of this vaccine such as inappropriate storage, poor preparation and/or administration of the vaccine. Therefore, it is very important to monitor vaccine take and seroconversion following vaccine application in the hatchery. Real-time PCR has been demonstrated to be a sensitive means to assess the replication of rHVT-F in the spleen of broilers and layers at around four weeks of age.

Antibody response can be measured by HI and ELISA tests, however, the former test has been shown to be more sensitive than ELISA in detecting early seroconversion.

Control of CAV

Prof. Harold Toro from Auburn University in the USA, who had been brought to the meeting by Lohman Animal Health, looked at how important vaccination and monitoring of breeder flocks is for the effective control of chicken infectious anaemia (CAV) in final generation birds.

He highlighted how the control of CAV in commercial broilers and layers is largely based on generating immune responses in the breeders via vaccination or natural infection as antibody positive breeders are unlikely to transmit CAV to their offspring.

In addition, maternal antibodies transferred to the offspring provide complete protection against CAV challenge during their first two to three weeks of life. Because breeders will be inevitably exposed to CAV challenge, some operations rely on natural infection for the purpose of eliciting



Attentive delegates at the recent WVPA Asia Meeting on Testing & Monitoring.

immune responses. However, this theoretically sound concept often produces suboptimal results in the field.

A reduced percent of antibody negative breeders may become infected on or after the onset of lay.

Vertically infected chickens and chickens infected horizontally due to low levels of maternal immunity will contribute to economic losses during production.

Evidence supported by data obtained from vaccinated flocks indicates that vaccination against CAV significantly reduces the percent of antibody negative breeder hens and the risk of reduced performance and health of broiler progenies.

Differentiation of mycoplasmas

Pablo Lopez from IDEXX Laboratories then looked at the differentiation of pathogenic avian mycoplasmas by real time-PCR. He concluded that the use of this technology for the detection of pathogenic avian mycoplasmas provides an excellent tool for flock monitoring, and can improve laboratory efficiency and provide economic and rapid results.

Commercially available reagents, specific for the detection of *Mycoplasma gallisepticum*, *synoviae* and *meleagridis* provide excellent sensitivity and specificity when compared to standards of testing currently used.

While ELISA technology remains the current standard in screening assays for avian mycoplasmas, it is hoped that these standardised reagent sets will improve testing confidence and be incorporated into current laboratory protocols as a robust and economical alternative to current methods of confirmatory testing.

Andreas Herrmann from Merial gave an update on the practical use of PCR tools for poultry disease diagnostics. He cited the good example of the use of PCR in breeders for *Mycoplasma gallisepticum* detection.

The pathogen is vertically transmitted from parents to progeny and represents a high sanitary risk for poultry production.

Such a routine or punctual monitoring introduces a better way to investigate the profiles of possible contamination throughout the growing period, and then in production.

Sampling is performed using swabs;

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trachea mucosa may be the target tissue to recover. Results may be generated quickly within a day after receiving the samples at the laboratory.

The implementation of this repeatable technique and then the analysis of data stored in PCR result database enable proper decision making in the context of MG control in breeder flocks, and allow for providing a 'free of MG status' for example, when needed, or when possible.

Another area of investigation in which PCR could be used is for the differentiation of vaccine strains from circulating pathogens that can be found in the field.

To differentiate a live vaccine from a

Asia	AFI	ZEN	DON	FUM	OTA
No. of tests	1620	1656	1674	1582	1593
Percent positive (%)	28	50	60	50	20
Average of positive (µg/kg)	53	149	752	1286	6
Maximum (µg/kg)	1563	5324	29267	26828	260

Table 1. Overview of survey results in Asia in 2013 (AFL = aflatoxin, ZEN = zearalenone, DON = deoxynivalenol, FUM = fumonisin B1 and B2, OTA = ochratoxin A.

pathogen is relevant in order to establish the most accurate diagnostic as possible. Live respiratory vaccines commonly used for infectious bronchitis and Newcastle disease are not currently easily differentiated

from the circulating pathogens. The use of differentiating PCR techniques, and the definition of vaccine strain genetic markers, may change the picture. More accurately, vector vaccines with their proper genetic construct, as for example the herpesvirus of turkey virus expressing different other pathogens' genetic sequences (infectious bursal disease for example) unique to each of them, may be easily differentiated from any wild pathogen.

Asian Avian Pathology lecture

Prof. M. R. Islam from The Agricultural University in Bangladesh then presented the Asian Avian Pathology Lecture which was entitled 'Infectious bursal disease: prophylaxis, detection and monitoring'.

Among the many things he highlighted was that maternally derived antibodies can protect chicks during the early days of life, but they may also interfere with the uptake of live vaccines in chicks.

Immune complex vaccine has been developed to overcome this problem. Recently developed HVT-IBDV vectored vaccine can immunise chicks in the presence of maternally derived antibodies.

Reverse transcription-polymerase chain reaction, coupled with restriction enzyme analysis or direct sequencing, and monoclonal antibody based antigen capture enzyme-linked immunosorbent assay are used for detection and characterisation of IBDV pathotypes and antigenic subtypes.

Indirect ELISA is used for serological profiling of breeder flocks for infectious bursal disease virus antibody titre with the objective of maintaining a uniform high level of maternally derived antibody in progeny chicks.

Gumboro disease vaccine

Roberto Soares then discussed field and laboratory approaches to monitoring Gumboro vaccines' 'take'.

He concluded that Gumboro disease control (prevention + protection) can be achieved by a complete colonisation of the bursa of Fabricius by a live attenuated vaccine strain. The assessment of vaccine 'take' is normally done by serology (ELISA), which demonstrates that the bursa was effectively colonised. Since ELISAs can not distinguish between antibodies induced by

vaccine and field Gumboro disease virus, other assays, such as histopathology and PCR, help to demonstrate the colonisation and confirm the strains that colonised the bursa of Fabricius respectively.

Mycotoxins in Asia-Pacific

Randy Payawal from Biomin then looked at testing and monitoring for mycotoxins in the Asia-Pacific region in 2013 (see Table 1).

He concluded that five groups of mycotoxins were highly prevalent in the feed ingredient samples from Asian countries. Scientific research has shown that no single strategy could control these multiple mycotoxins.

No mycotoxin binder could bind Fusarial mycotoxins, such as deoxynivalenol, T-2 and zearalenone, very well. Mycofix protects animals from a wide range of adsorbable and non-adsorbable mycotoxins, by incorporating three strategies – biotransformation, adsorption and bioprotection.

Poultry house dust

On the second day short papers covering active research in the region were presented, of which some were pertinent to breeders. The day started with three papers from Australia on screening poultry house dust for viral pathogens.

Testing from representative individual chickens is cost prohibitive and generally involves temperature sensitive samples that require maintenance of cold chain to and within the laboratory. Monitoring of pathogen loads in poultry house dust is an attractive alternative. The dust produced within poultry houses is a mixture of feather dander, and powdered faecal, litter and other material, including pathogens shed from the chickens. It is dry so does not decompose at room temperature and can therefore be transported in the normal mail system.

Furthermore, it is non-invasive to collect, and is broadly representative of the entire housed flock. Virus may be isolated or sequenced from the dust material. We have shown that monitoring of Marek's disease virus in broiler house dust samples using real-time quantitative PCR (qPCR) is an effective disease control tool in Australia where it is commercially implemented.

As Marek's disease virus is shed in dander, it is an obvious candidate for this approach. Investigations of this method have now been extended to faecally shed fowl adenoviruses, infectious bursal disease virus, chicken infectious anaemia virus and in the control of the sanitary status of breeder flocks

Results to date indicate that all of these viruses are readily detected and enumerated by qPCR of dust collected from infected chickens and thus this method has potential for routine surveillance of these viruses.

Mazhar Khan and colleagues from the University of Connecticut in the USA highlighted that designing a vaccine against avian influenza (AI) is challenging due to the highly mutable genome of that virus.

Peptide nanoparticle technology has been demonstrated as a powerful platform for the delivery of epitopes to elicit strong immune response, providing promise in the development of universal influenza vaccines.

Particularly, in this study, four self-assembled nanoparticle peptides (SAPNs) were designed to organise AI M2e peptide as a tetramer and, in addition, to include conserved helices A, C and the cleavage peptide (CPP) of haemagglutinin as well as chicken specific T cell (HTL) epitope, respectively in each construct.

Structural organisations of the epitopes on the SAPNs are to best display them in their native conformation as in the virus to induce immune response.

Groups of specific pathogen free (SPF) chickens were immunised intramuscularly with these four constructs for three times with two weeks apart between each vaccination. ELISA results suggesting that tetra-M2e-HTL and tetra-M2e-Helices A construct induce strong antibody titre in serum of vaccinated chicken compared to the control group.

H7N9 in China

Yang Yu from Zhejiang University in China reported on the detection and isolation of H7N9 avian influenza virus and its prevalence in China

The first outbreak of H7N9 virus was in eastern China in March 2013. This virus had caused a number of cases of infection and death. The frequent reassortment of influenza A virus resulted in the emergence of a novel H7N9 virus. Until now, H7N9 virus is still sporadic in China.

One of the characteristic features of influenza A virus is its segmented genome, which allows for exchange of eight gene segments between different virus strains. Genetic reassortment among different co-infected viruses may generate novel human adapted virus with drastic antigenic changes or antigenic shift.

To investigate the origins of H7N9 that caused human infection, nine specimens were collected from trade markets in Hangzhou, the capital of Zhejiang province. One of them was from quail, one was from duck, and the others were chicken specimens.

The following methods were conducted for detecting the specimens: haemagglutinin inhibition, specific primers for RT-PCR, detection of avian influenza virus H7N9 subtype RNA (PCR-Fluorescence Probing), which was developed by Zhejiang CDC, and high throughput next generation sequencing.

Finally, six were detected as H7N9. Surprisingly, coexistence of H7N9 and H9N2 was confirmed in some specimens. ■