

Molecular Biology and Pathogenesis of Avian Viruses

POSTER ABSTRACT BOOK

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Poster Number: 01

Determining the dynamics of IBDV replication organelle movement *in vitro* using a split-GFP tagged virus.

Abstract

Infectious bursal disease virus (IBDV) is a highly contagious immunosuppressive infection of chickens that causes production losses to the poultry industry. Despite its importance, little is known as to how it replicates within the host cell. Fluorescently tagged reporter viruses are important tools for studying the interaction of viruses with host cells. Using a reverse genetics system designed for the IBDV strain PBG98, we have tagged a small sub-unit of Green Fluorescent Protein, GFP11, to the IBDV VP1 protein to make a split GFP virus (PBG98-VP1-GFP11). When DF-1 cells were transiently transfected with the rest of the GFP molecule (GFP1-10) and subsequently infected with PBG98-VP1-GFP11, infected cells had multiple green foci in the cytoplasm. When cells were fixed and stained with antibodies against dsRNA and the VP3 protein, there was a high degree of co-localisation, suggesting these foci are Replication Organelles (ROs). When imaged from 12 hours to 24 hours post-infection, the average diameter of the foci increased while the average number of foci per cell decreased, consistent with the coalescence of multiple ROs over time. When movies were taken of the infected cells at 18 hours post-infection for a period of 2 hours 30 minutes, small foci moved in the cytoplasm and some coalesced, whereas larger foci remained more static. Taken together, the PBG98-VP1-GFP11 virus will be a useful tool to further elucidate IBDV-host cell interactions.

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Poster Number: 02

Survival kinetics for Newcastle disease viruses (NDV) using virulent and avirulent avian avulavirus type-1 strains (AAvV-1, formerly avian paramyxovirus type-1 [APMV-1]) for modelling poultry industry parameters

Abstract

Virus survival variability was determined for six Newcastle disease isolates, virulent AAvV-1 viruses for representative lineages-genotypes: 1) PPMV-1/partridge/Scotland/7575/2006 (4b-VIb); 2) APMV-1/chicken/Bulgaria-NDV-3-112/2013 (5a-VIIa); 3) APMV-1/chicken/England/1453/1996 (5b-VIIb); 4) APMV-1/chicken/Nepal/8-43/2010 (5b-VIIb); 5) APMV-1/chicken/Israel/380/2005(5d-VIIId) and one avirulent isolate 6) APMV-1/chicken/N Ireland/1/1967 (1-I) at different temperatures (4°C, 20°C, and 30°C), on poultry house surfaces (plastic, metal, wood) at 21°C and in vivo derived environmental samples.

From virus survival experiments using Madin-Darby bovine kidney cells, we found variation in DT (time for virus titre to reduce by 1 log₁₀ at temperature (T)); at D4 data ranged from 3-50 days; D20 from 2.4-13.3 days and D30 from 1.4-11.5 days. On poultry house surfaces, viruses 1/2/3 survived hours on plastic and metal, but under one hour on wood.

Drinking water from rooms with APMV-1/chicken/Bulgaria/112/2013 (5a/VIIa) challenged pheasants showed positive PCR results from days 8-10 and in faeces at 14 dpi; from challenged partridges no viral RNA was detected in drinking water, but day 7 faeces was strongly positive.

Environmental survival data for NDV at temperatures, representing UK winter, summer and heated sheds, provides evidence data to supports statutory epidemiological investigations; indicating possible routes of virus introduction onto farms and dissemination through poultry housing and can contribute towards reducing financial and animal welfare burdens to industry and government.

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Poster Number: 03

The application of CLIPS Precision Epitope Mapping to identify immunogenic epitopes on the S1 of Infectious Bronchitis Virus strains M41, 4/91 and QX

Abstract

The major surface protein of IBV, spike, is highly diverse between serotypes and amino acid differences in the S1 sub-unit are thought to be responsible for the poor cross-protection given by vaccination. The level of amino acid homology between the S1 of serotypes does not correlate to the level of cross-protection, as some strains of low homology can confer high levels of cross-protection, suggesting there may be a conformational element associated with cross-protection. Here, we attempt to address the issue of poor and unpredictable cross-protection in the field by using a novel epitope mapping technology to identify immunogenic epitopes present on S1 of M41, 4/91 and QX strains. Homologous and heterologous-vaccination challenge trials with rIBV were conducted and used as a tool to generate isogenic polyclonal serum to identify epitopes. Briefly, CLIPS epitope mapping used high-throughput microarrays of overlapping surface-immobilised constrained peptides (looped and linear) covering the S1 sequences of M41, 4/91 and QX to produce functional mimics of complex binding sites. The antibody binding affinity to each peptide construct was determined and a panel of linear and conformational epitopes were identified containing both serotype-specific and shared sequences. Cluster analysis showed a degree of comparable binding profiles between serum from heterologous vaccinated groups highlighting recognition of shared epitopes. Structural modelling of spike proteins has been used to visualise epitope locations. A panel of epitopes have been selected to test proof-of-principle for generation of rIBV expressing a spike with chimeric epitopes.

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Poster Number: 04

Iranian H9N2 Avian Influenza Virus is closely related to Pakistani Parakeet

Abstract

Sequence analysis and phylogenetic study of hemagglutinin (HA) gene of H9N2 subtype of avian influenza virus isolates (outbreaks of 1998-2002) in Tehran province (Iran) were studied. PCR products of a 430-bp fragment of 16 isolates were sequenced and then were aligned with the reported sequences in Genbank. Nucleotide sequence comparisons of HA gene from Iranian isolates showed 97-99% identity within the group, and 98% homology with the two isolates [A/Parakeet/Narita/92A/98 (H9N2)] and [A/Parakeet/Chiba/1/97 (H9N2)] from Pakistani parakeets imported to Japan. On the basis of phylogenetic evidence and even sequence comparison of Neuraminidase (N) gene of [A/Parakeet/Chiba/1/97 (H9N2)] and [A/Chicken/Iran/IT/99 (H9N2)](Li,2002) it is proposed that the emergence of H9N2 avian influenza infection in Iran originated in Pakistan. Due to the high percentage of H9N2 homology isolates of Iran with other isolates, namely A/quail/HongKong/G1, in Genbank and based on published reports for high similarity with infecting human H5N1 isolates, it seems that the potential of Iranian avian influenza isolates to infect human should be considered.

Key Words

Avian influenza (AI), Hemagglutinin (HA), Neuraminidase (N), H9N2 subtype, Phylogeny

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Poster Number: 05

Genetic diversity of avian paramyxoviruses serotype 1 circulating among poultry and wild birds in Kazakhstan

Abstract

The several transcontinental flyways of migratory birds overlap and pass through the territory of Kazakhstan. Virological surveillance of avifauna in this region is important for study of genetic diversity of avian paramyxovirus serotype 1.

Tracheal and cloacal samples from wild birds were collected using sterile swabs placed in viral transport medium. Samples were inoculated into 10-day-old embryonated chicken eggs and reverse transcription PCR (RT-PCR) assays were performed via a one-step protocol. The PCR products were sequenced and phylogenetic trees were constructed using the 'Neighbour Joining' method.

According to the results of phylogenetic studies of fusion protein gene, APMV-1/chicken/Almaty/36/2016 and APMV-1/rock pigeon/Chokpak/6444/2015 was classified as different classes (1,2). The Kazakhstan strain APMV-1/chicken/Almaty/36/2016 was in the group of viruses belonging to the VII genotype of class 2, and the isolate APMV-1/rock pigeon/Chokpak/6444/2015 was included in the cluster within the class 1. Velogenic variant APMV-1/Cormorant/Alakol/6946/2016 is referred to the genotype Vb, the other - APMV-1/common myna/Chokpak/6434/2015 to genotype VI.

Analysis of the amino acid (aa) sequences of HN and F proteins of the Kazakhstan isolate APMV-1/chicken/Almaty/36/2016 revealed the presence of the basic aa RRGKR at positions 112-116 of the F-protein cleavage site, which demonstrates their high pathogenicity. The APMV-1/rock pigeon/Chokpak/6444/2015 do not bear multiple basic aa in the indicated site (ERQER). These data demonstrate a genetic diversity of avian paramyxoviruses serotype 1 circulating among poultry and wild birds in Kazakhstan.

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