Detection of infectious bursal disease virus serotype 2 (IBDV-2) in New Zealand poultry and waterfowl

Summary

Investigations were carried out on three unrelated free-range chicken broiler farms after IBD antibody ELISA reactors were detected via the poultry industry sero-surveillance programme. The investigation was part of ongoing efforts to inform and validate New Zealand's IBD surveillance programme. The investigation excluded the presence of pathogenic IBD serotype 1 viruses (IBDV-1) but identified a non-pathogenic IBDV serotype 2 (IBDV-2) virus, which was also identified in wild mallard ducks (*Anas platyrhynchos*). The investigation concluded that IBDV-2 was the most likely cause of the sporadic low-level seropositivity seen during the programme. These findings support New Zealand's claim that Gumboro disease (also known as infectious bursal disease, IBD) is absent from commercial poultry, and will pave the way for the development of serotype-2-specific serological and molecular assays. Such tests will enable the rapid exclusion of IBDV-1 in poultry flocks identified with serum reactors through the ongoing IBD sero-surveillance programme.

Introduction

Infectious bursal disease (IBD – avibirnavirus) or Gumboro disease is an acute, highly contagious viral infection of young chickens that can cause immunosuppression resulting in morbidity and mortality from secondary infection. The IBD virus is characterised by a bi-segmented, double-stranded ribonucleic acid (dsRNA) genome consisting of two segments (A and B). The virus has no envelope, a simple icosahedral capsid structure, and a diameter of 58–60 nm. This relatively simple structure renders the virus very environmentally resistant (Eterradossi & Saif 2013).

Two IBDV serotypes (1 and 2) have been identified, differentiated in vitro by the lack of significant cross-neutralising antibodies, and in vivo by the absence of cross-protection (van den Berg et al. 2000). Serotype 1 strains are pathogenic in chickens, with strains classified according to virulence into various pathotypes: sub-clinical (scIBDV), classic virulent (cvIBDV) and very virulent (vvIBDV). In other avian species serotype 1 is avirulent (Oladele et al. 2009; McFerran et al. 1983; Eterradossi & Saif 2013). Serotype 2 strains do not cause disease in any birds. Turkeys are considered the natural host of serotype 2 viruses, although these may also be isolated from other birds (Eterradossi & Saif 2013). Although IBDV serotypes 1 and 2 have been detected in waterfowl and other wild birds, neither has been confirmed as a cause of disease (Wilcox et al. 1983; Jeon et al. 2008; Kasanga et al. 2008).

In New Zealand, an IBDV serotype 1 virus was identified in clinically normal commercial poultry in 1993. Investigations concluded that the virus was most likely to have been introduced in a contaminated or mislabelled vaccine (Thompson 1994; Motha 1996; Ryan et al. 2000). The poultry industry subsequently took steps to eradicate infection by imposing movement controls and decontamination protocols on all farms that tested positive in an ongoing sero-surveillance programme. The last IBD-seropositive flock was detected in January 1999 (Ryan et al. 2000).

Since that time, the New Zealand poultry industry has operated a "Country Freedom Quality Plan" for IBD surveillance and accreditation of commercial flocks (Brooks 2003; Ryan et al. 2000; Mulqueen 2018). Serum reactors in the ELISA run through the commercial poultry laboratories are referred to the MPI Animal Health Laboratory (AHL) at Wallaceville for the virus-neutralisation test (VNT). MPI's Incursion Investigation Team, in collaboration with the AHL, has investigated a number of flocks in which serum reactors in the screening ELISA have produced low-titre (non-specific) reactors in the VNT. In all cases, investigations have excluded IBDV (Bingham et al. 2006; Bingham et al. 2010).

This report describes the key features of an investigation into historical samples taken in 2015–2016 from three free-range broiler farms with low-level seropositivity. The investigation was carried out as part of ongoing efforts to inform and validate the active surveillance programme and demonstrate that Gumboro disease is absent from New Zealand.

Broiler farms investigated

Investigations were carried out on three unrelated free-range chicken broiler farms (Farms 1-3, **Figure 1**) during 2015–2016, after the detection of IBD ELISA (IDEXX FlockChek IBDV, Maine, USA) reactors through the poultry industry surveillance programme. Low-titre serum reactors were confirmed in the serotype 1 IBDV VNT carried out at the AHL. The VNT used the New Zealand IBDV-1 virus isolated in 1993, and followed the test procedure detailed in the OIE Terrestrial Manual Chapter 2.3.12 (Motha 1996; OIE 2016).

Figure 1: Barn and outdoor run set-up at one of the free-range chicken broiler farms investigated

After confirmation of low-titre positive reactors in the VNT, the Incursion Investigation Team carried out a standard investigation into the clinical and pathological presentations, and laboratory findings evident on each farm, with the aim of determining the cause of the serum reactors and, importantly, establishing whether IBDV was present.

The farms all had healthy flocks (assessed throughout the ~6-week production cycle) with no clinical signs, increased mortality or pathology indicative of IBDV infection. Mortality figures were within industry standards, with all barns on all farms demonstrating excellent performance and low mortality figures. No differences in mortality were evident between barns with and without IBD serum reactors. Cumulative mortalities over the ~35-day production cycle for barns with and without serum reactors were 2.1–4.0 and 1.9–6.5 percent respectively.

No IBDV was suspected, given the lack of clinical signs and pathology, and follow-up work was aimed at understanding the cause of the serum reactors, to help inform the surveillance programme. A cross-sectional serological survey of all barns (~25 sera per barn) on every farm was carried out, and cloacal swabs and fresh and fixed tissues, including bursa, spleen and caecal tonsil, were collected on-farm and/or at processing.

Serum samples were tested by ELISA, with reactors detected inconsistently across the barns (**Figure 2**). The VNT, carried out on ELISA-positive sera (S/P > 0.2), returned titres predominantly ≤ 1:16 (though occasional higher). Histopathology of bursas from seropositive flocks was mostly within normal expected limits, with occasional bursas showing one or more changes including mild-to-moderate lymphocyte depletion, cryptosporidiosis or focal bacterial-associated heterophilic inflammation, and rare peri-follicular haemorrhagic change (thought to be consistent with electrical stunning prior to slaughter). There was no evidence of generalised necrosis and atrophy of bursal follicles as would be expected from pathogenic IBD infection. An RT-qPCR assay for IBD serotypes 1 and 2 (Hein & Trinidad 2006) produced positive results, indicating the potential involvement of an IBD-like virus.

Virus isolation was attempted (five serial passages) on positive tissue samples identified by IBD RT-qPCR using three cell lines and CAM inoculation of embryonated chicken eggs. Despite intensive attempts, no birnavirus was isolated as assessed by molecular assays run after each passage. This was not unexpected, as isolating IBD field viruses in vitro is often challenging (Soubies et al. 2018). A reovirus (endemic virus) was isolated in some samples and was confirmed by electron microscopy and PCR.

Molecular investigation of PCR-positive samples

To enable confirmation and further characterisation, samples that tested positive by the IBD RT-qPCR were examined using additional primer sets and whole genomic sequencing. Conventional PCRs included one targeting segment A VP2 gene (Eterradossi et al. 1998; Wu et al. 2007) and the other segment B VP1 gene (Le Nouën et al. 2006; Wu et al. 2007). Procedures followed the OIE Terrestrial Manual Chapter 2.3.12 (OIE, 2016). Only primers for the B segment produced PCR product (549 bp specific for IBDV sequences). This nucleotide sequence when blasted had up to 92 percent nucleotide identity to both serotype 1 and 2 IBD viruses (no discrimination) in GenBank. This result was consistent across all three farms.

Given that the primer sets targeting segment A failed to produce a signal, a number of primer sets were specifically designed, based on IBDV genome sequences of the serotype 1 and 2 viruses deposited in GenBank. Using this approach, bursas from the most recently affected farm (farm 3) were used to sequence the full genome of segment A (3,243 nucleotides). When blasted in GenBank this sequence had 88–89 percent nucleotide identity to IBDV serotype 2, and 84 percent identity to IBDV serotype 1 viruses. Partial sequencing of positive bursal tissue from the first two farms aligned with findings for Farm 3.

These results were considered to have excluded IBD serotype 1 viruses, and specifically the IBD-1 virus that entered New Zealand in the early 1990s. Findings appeared to indicate the presence of a poorly characterised avibirnavirus. The OIE IBD Reference Laboratory (Ploufragan-Plouzane, France) was engaged to assist with further characterisation of the virus (see below).

Wild waterfowl tested

As part of the investigation into potential sources of virus, cloacal swabs collected from wild mallard ducks (*Anas*

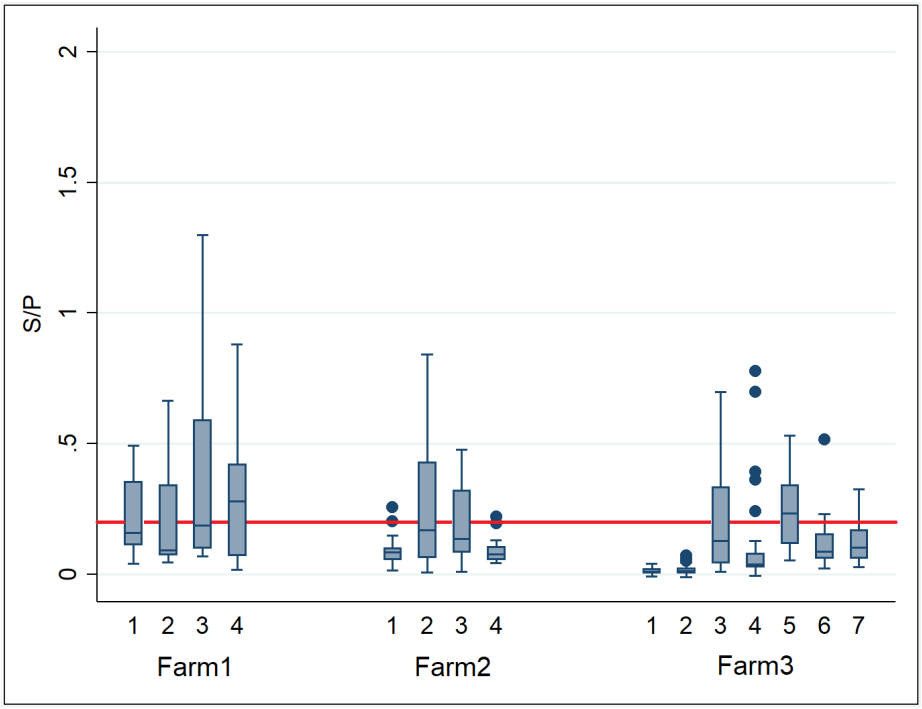

Figure 2: Box and whisker plots showing IBDV ELISA (IDEXX FlockChek IBDV) sample-to-positive (S/P) ratios from a cross-sectional sero-survey of individual barns on three farms. The red horizontal line indicates the manufacturer's cut-off at 0.2. The boxes span the interquartile range of values (25–75 percent), with the central line indicating the median value. The whiskers extend to 1.5x the interquartile range.

platyrhynchos) during MPI's Avian Influenza Surveillance Programme were tested using the PCR protocol and sequencing described above (Stanislawek et al. 2012). Samples were collected in the summer months (January–March) of 2016 from three locations in the North Island (Waikato, Bay of Plenty and Hawke's Bay. A low prevalence of PCR-positive cloacal swab samples was detected in all three areas: 3/320, 1/80 and 2/129 respectively. Positive samples were confirmed by sequencing undertaken at the AHL, where the analysis indicated a high similarity to the virus detected in the poultry broiler farms.

Confirmation of IBDV serotype 2

New Zealand poultry sequences from farms 1–3 were submitted to the OIE IBD Reference Laboratory (Ploufragan-Plouzane, France) and aligned with sequences from IBDV strains representative of the genetic diversity for IBDV (Abed et al. 2018). Phylogenetic analysis of segment A by neighbour joining revealed that the New Zealand sequence unambiguously clustered together with European (strain 23/82) and American (strains OH and Turkey/PA/00924/14) strains of serotype 2, although they also exhibited significant genetic differences consistent with the 88–89 percent identity reported above. Sequence examination of the VP2 gene revealed an insertion at position 249, which is considered typical for serotype 2 viruses. The submitted New Zealand sequences reveal new genetic data on serotype 2 viruses and are of interest to the scientific community.

Conclusion

Consistent with other reports, we conclude that a non-pathogenic serotype 2 IBDV is the most likely cause of the sporadic low-level serum cross-reactivity seen during New Zealand's IBD surveillance programme (Ashraf et al. 2006). Although it is not possible to definitively explain the origin of the virus in the broiler farms, phylogenetically similar serotype 2 IBD viruses were diagnosed in both the free-range broilers and wild New Zealand mallards. It is reasonable to expect the occasional introduction of IBD-2 from wild birds, especially under a free-range system.

The investigation described here demonstrates New Zealand's continued efforts to validate its claims that IBD serotype 1 virus is absent from New Zealand (Herrera 2019). Work underway will see further attempts to isolate the New Zealand serotype 2 virus, working in collaboration with the OIE Reference Laboratory. This work will pave the way to development of serotype-2-specific serological and molecular assays to help with the rapid exclusion of IBDV-1 (and confirmation of the involvement of IBDV-2), in future cases of serum reactors detected through the ongoing IBD sero-surveillance programme (Ryan et al. 2000, Mulqueen 2018).

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