Epidemiological, experimental and diagnostic investigations into an acute paralysis syndrome of broiler chickens in Australia

by

Brendan Sharpe

B.V.Sc. (Hons I) (University of Sydney, Australia)

a thesis submitted for the degree of

Doctor of Philosophy

in Animal Science, School of Environmental & Rural Sciences,
University of New England, Australia

January 2016.

Abstract

An acute paralysis syndrome (APS) of broiler chickens was first reported in 2010 in an Australian chicken meat production region. The APS was characterised by flaccid paralysis of the neck, prostration and eventually death of the affected chicken and elevated flock mortalities from 26 days of age. The purpose of the work presented in this doctoral thesis was to establish causation of the APS, risk factors for it and management strategies for controlling it. An initial review of literature (Chapter 2) enabled a broad differential diagnosis list to be devised in consideration of the potential causes of the APS. The collation and synthesis of diagnostic tests performed on field cases (Chapter 4) highlighted the need for further diagnostic testing to assess for the presence of serotype-1 Marek's disease virus (MDV1), avian encephalomyelitis virus (AEV) and flaviviruses in affected chickens. The main pathological findings from field cases were that 32 % of affected chickens displayed vasculocentric encephalitis focussed in the cerebrum. Neurological examination of affected chickens localised lesions to within the forebrain, consistent with the observed brain histopathology. Exclusion testing ruled out Newcastle disease and avian influenza and serum ELISA assays for *Clostridium botulinum* toxins C and D were consistently negative. MDV1 load in shed dust revealed very high counts of MDV1 in sheds in the affected region prior to the implementation of Marek's disease (MD) vaccination, after which a sharp reduction in MDV1 load in shed dust was observed. Despite this the APS continued to occur and MDV1 continued to be found in a small proportion of APS affected and MD vaccinated chickens.

A retrospective case-control study of farms affected with the APS was performed (Chapter 5) in order to describe the temporal and spatial distribution of the APS, define the impact of the APS on flock performance, identify risk factors which were associated with the APS and to devise mitigation strategies which may be implemented on farm. The APS caused bird losses but did not reduce growth rates. Flock wastage (mortality + culls) was significantly greater ($P < 0.0001$) for flocks with the APS (15.9 ± 0.6 %) than for flocks without the APS (5.5 ± 0.2 %). The majority of wastage in affected flocks occurred from 26-51 days of age. Mean bodyweights of flocks with the APS were significantly greater than flocks without the APS at 14, 21 and 28 days of age.

Multivariate logistic regression analysis showed that the most important risk factor for the APS was mean daily maximum shed temperatures from hatch to 7 days of age below the Ross 308 guidelines (odds ratio 3.99 per unit of 1.0°C, $P<0.002$). Other risk factors relevant to APS mitigation included: bodyweights above the Ross 308 breed guidelines between 14 – 28 days of age, maximum shed temperatures exceeding the Ross 308 breed guidelines from 15 days of age, excessive fluctuations in shed temperatures and increased flock age at first thinning-out.

The APS was experimentally reproduced (Chapter 6) in broiler chickens inoculated at 21 days of age with spleen cells (35% of chickens) or whole blood (35% of chickens), and in chickens exposed to contaminated litter from 21 days of age (10% of chickens). The APS was not reproduced in broiler chickens inoculated with brain tissue or sham-challenged at 21 days of age, in broiler chickens challenged at hatch with any of the challenge materials or in specific pathogen-free layer chickens challenged at either age with any of the challenge materials. The pattern of reproduction confirmed an infectious aetiology (at least in part) of the APS and additionally suggested that maternal antibody may be protective against the APS from early challenge. Affected chickens demonstrated an identical clinical syndrome to affected field chickens. In broiler chicken groups challenged at 21 days of age, males had a significantly increased ($P = 0.02$) mortality compared to females confirming a greater susceptibility of males to the APS. The effect of MD vaccination on mortality was not significant. Histopathology of the brain was consistent with field findings. Polymerase chain reaction assays did not detect evidence of MDV1, AEV or flaviviruses in affected chicken tissue. Experimental reproduction of the syndrome was not achieved in three of four subsequent experiments (Chapters 7-8) and the most plausible reason for the difficulty in reproduction was due to low growth rates achieved in these subsequent experiments.

Whole genome next-generation sequencing (NGS) and subsequent bioinformatic analysis was performed from DNA and RNA extracted from spleen, blood and brain tissue from APS affected and clinically normal chickens in an attempt to identify infectious agents present in affected chickens and absent in normal chickens (Chapter 9). Evidence of feline herpesvirus 1 in 3/7 affected chicken DNA samples and respiratory syncytial virus in 3/6 affected chicken RNA samples were found.

In summary the APS is caused at least in-part by an infectious agent/s due to the pattern of reproduction demonstrated and the associated pathology. Various management strategies are recommended for reducing the incidence of the APS on farms including: maintaining shed temperatures to the guidelines recommended for the Ross 308 breed throughout the life of the flock but particularly within in the first 7 days of age, ensuring growth rates are within the breed guidelines and avoiding delays in flock thinning-out. Recommendations for future work include refining of the experimental reproduction model focussing on achieving adequate growth rates to allow disease manifestation, more extensive epidemiological analyses, testing any protective effect of early exposure to the putative agent/s by re-using litter on-farm and following up on NGS results utilising polymerase chain reaction based assays.

Certification

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis and all sources used have been acknowledged in this thesis.

……..

Brendan Sharpe

January, 2016

Acknowledgements

Firstly, sincere thanks to my principal supervisor Professor Steve Walkden-Brown for the expertise, mentorship and friendship that he has provided throughout this study. Steve encouraged me to not lose sight of the 'journey' that is a PhD whilst keeping clear outcomes in mind, which allowed me to complete my investigations in a timely manner and gain much more than what is presented in this thesis. To my co-supervisors Professor Margaret Katz, Professor Peter Groves and Dr Katrin Renz, thank you for you generous provision of time and expertise and the patience you have extended to me throughout this program. Thanks also to Dr Rodney Reece for his pathological expertise and more importantly his mentorship.

To my post-graduate colleagues Robin Achari, Sithara Ralapanawe, Mamdouh Alsharari, Kanchana Jayasundara and Salih Wajid; thank you for the assistance and friendship that each of you have provided throughout. Expert advice and technical assistance provided to me by Chris Morrow, Dieter Bulach, Fakhrul Islam, Sheridan Alfirevich, Sue Sharpe and technical staff within the School (Sue Burgess, Michael Raue, Andrew Blakely and Graham Chaffey) has been essential and I thank each for their generous provision of time.

I acknowledge research funding provided by the Australian Poultry CRC and the University of New England. Additional thanks for the support provided by staff within the Poultry CRC (Professor Minghan Choct, Liz Roan, Michael Warner and Lloyd Thompson) and within the School of Environmental and Rural Science (Frank Leayr, Shirley Fraser and Jo Porter).

Finally, I wish to thank my parents who have supported me right throughout my education and encouraged me to maintain focus. To Kate, thank you for the love and support that you have provided me with throughout this journey and the sacrifices that you have made.

Table of Contents

List of Figures

List of Tables

List of Publications

Materials from this thesis have been published in the following conference papers.

1. Sharpe, Brendan; Katz, Margaret; Reece, Rodney; Renz, Katrin and Walkden-Brown, Steve. Investigations into an acute paralysis syndrome of broiler chickens. *Australasian Veterinary Poultry Association*, Gold Coast QLD, May 2014.

2. Sharpe, Brendan; Groves, Peter; Katz, Margaret; Reece, Rodney; Renz, Katrin and Walkden-Brown, Steve. Epidemiological and experimental investigations into an acute paralysis syndrome of broiler chickens in Australia. *British Veterinary Poultry Association Spring Meeting*, Harrogate UK, March 2015.