Australasian Veterinary Poultry Association

Scientific Meeting

Gold Coast Convention and Exhibition Centre

Queensland

May 2010

WEDNESDAY 26th MAY		
Time	**Speaker**	**Chair: Tom Grimes**
8.45 – 9.00	Ben Wells	Opening
9.00 – 9.30	Ton Schat	Avian Influenza: It is Still a Problem in Three Continents
9.30 – 10.00	Kathy Gibson	Avian Influenza Vaccination – Preparing For an Outbreak
10.00-10.30	Clive Jackson	The National Newcastle Disease Management Plan - 2002 to 2012
10.30 – 11.00	**Morning Tea**	**Chair: Peter Scott**
11.00-11.30	Viv Kite	Report on the Activities of the International Poultry Council
11.30 – 11.50	Tom Grimes	Poultry Disease and Control in Overseas Countries – Relevance to Australia
11.50-12.10	Hayley Blacker	An Update of Recent Infectious Laryngotracheitis Virus (ILTV) Strains Identified in Australia
12.10-12.30	Branko Karaconji	Feather PCR Diagnostic Testing for Marek's Disease.
12.30 – 1.30	**Lunch**	**Chair:**
1.30 – 2.00	Pat Blackall	Of Rabbits, Chickens and Champagne – Everything Old is New Again
2.00-2.20	Marg Mackenzie	Salmonella and Campylobacter Control in Processing
2.20 – 2.40	Jan-Maree Hewitson	SNP Types of *Campylobacter jejuni* Isolated from Different Hosts
2.40 – 3.00	Tim Wilson	Evaluation of Residues in Eggs after Treatment of Pullets with Toltrazuril, Sulfadimidine or Amoxycillin.
3.00 – 3.30	**Afternoon tea**	**Chair:**
3.30 – 3.50	Pat Blackall	The Poultry CRC And RIRDC – Current Research Topics and Their Objectives
3.50 – 4.10	Hayley Blacker	Diagnostic Technologies Available to the Australian Poultry Industry
4.10 – 4.30	Penelope Steer	Current status of Inclusion Body Hepatitis and Fowl Adenoviruses in Australian Poultry
4.30 – 4.50	Charissa Smith	Modification of Laying Hen Responses to Fowl Pox with Homeopathic Medicines
5.00-6.30	**AVPA AGM**	
7 for 7.30	**AVPA dinner**	

THURSDAY 27th MAY		
Time	**Speaker**	**Chair: Clive Jackson**
8.30 – 9.00	Ton Schat	Chicken Infectious Anaemia Virus: An Insidious Problem for Conventional and SPF Poultry Producers
9.00 – 9.20	Tim Wilson	CAV Vaccination, Comparison with Natural Exposure in Broilers
9.20 – 9.40	Soy Rubite	Case Report: Avian Nephritis Virus (ANV) Infection in a Broiler Flock in Victoria
9.40 – 10.00	Kylie Hewson	Isolation of Avian Nephritis Virus from Australian Chicken Flocks
10.00 – 10.20	Peter Groves	A Survey of the Occurrence of "Spotty Liver Syndrome" In Commercial Layer Chickens
10.20 – 11.00	**Morning tea**	**Chair: Chris Morrow**
11.00 – 11.30	Donna Hill	Incubation, Embryology and the High Yield Embryo
11.30-11.50	Nalini Chinivasagam	Preliminary Trends From a Study on Food-Borne Pathogens and Litter Re-use Across Broiler Cycles
11.50-12.10	Steve Walkden-Brown	Towards Improved Inactivation and Monitoring of Viral Pathogens in Reused Broiler Litter
12.10-12.30	Amir Noormohammadi	Identification of Chlamydial Species in Chickens by PCR-HRM Curve Analysis
12.30	**Close**	
12.30 – 1.30	**Lunch**	

Avian Influenza: It is Still a Problem in Three Continents.

K.A. Schat

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Highly pathogenic avian influenza (HPAI) has attracted world-wide interest in first instance since 1996/97 and especially since 2003. The appearance of the Asian lineages of H5N1 HPAI virus (referred to in this paper as H5N1 HPAIV) has instilled wide-spread fear in many political, scientific and other circles that a deadly flu pandemic is at our doorstep. In 2009, this fear was replaced by the occurrence of H1N1 Mexican flu virus, which became the new pandemic flu virus and, as a consequence, the threat of a H5N1 HPAIV pandemic disappeared from the mainstream press. Although the pandemic has not (yet) occurred, H5N1 HPAIV has caused the dead of untold free-living birds and poultry since 2003 as well as a very low infection rate in humans albeit with a high fatality rate. In this review, I will first address the current information on the incidence of H5N1 HPAIV in humans followed by an update on the situation in free-living birds and poultry. Finally, I will discuss the possible reasons for the absence of this particular subtype of AIV in Australia. Detailed information on H5N1 HPAIV can be found in recent review papers on the ecology of AIV (4), outbreaks and biological properties (5), and pathogenesis with a focus on the disease in humans (7).

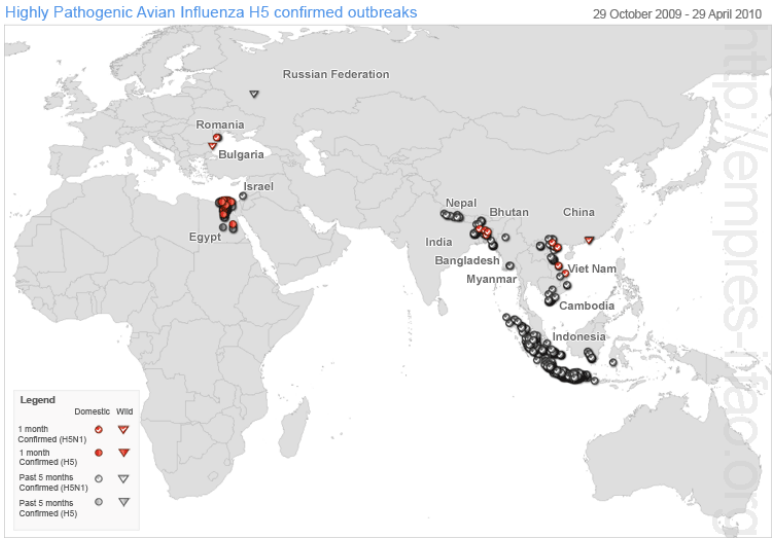
The incidence of H5N1 in humans has fluctuated from 2003 to May 6 2010 between 4 and 115 cases in 2003 and 2006, respectively. The total incidence remains low with 498 confirmed cases and 59% mortality (10). In 2010, the WHO reported thus far 30 cases in 4 countries (Cambodia, Egypt, Indonesia and Vietnam) with a 40% fatality rate. The majority of these cases (63%) occurred in Egypt (10). Clearly, the disease remains present in humans and the risks remain real that the virus mutates to allow sustained human-to-human transmission.

The situation in poultry and free-living birds is less clear than the overview for human cases. The OIE reports outbreaks in 51 countries representing 3 continents since 2003 with 19 countries reporting more than 20 outbreaks during this period (6). On the other hand, Sims and Narrod (9) mentioned that "some 60 countries" have been affected. During the period of October 29, 2009 to April 29, 2010 the FAO (1) reported outbreaks in 14 countries with many of these outbreaks occurring in South-east Asia, including Indonesia, and the Indian subcontinent (Fig. 1). The actual number of outbreaks is probably higher than the reported numbers and some countries may not report all outbreaks to the OIE. Intensive surveys of free-living birds have been conducted in America and Australia to determine if H5N1 HPAIV is present in birds. Thus far, this subtype has not been detected in America (4) and Australia (2).

In order to better understand the reasons why these two continents have remained free of H5N1 HPAIV it will be important to examine the ecology of avian influenza viruses. The major natural reservoirs of avian influenza viruses consist of two orders of birds: the Anseriformes and the Charadriiformes. The later order has 13 families comprising 91 genera with approximately 340 species. Important families in this group are the sandpipers and terns and gulls. These birds migrate often over large distances: for example the black-tailed and bar-tailed godwits may breed in the tundra's of Siberia and spend the northern winters in balmy Australia. The Anseriformes consist of 3 families with the Anatidae being the largest family consisting of 140 species of ducks, geese, and swans. The Anseranatidae consists of only one species: the Magpie Goose. In contrast to Europe, Africa and the Americas where ducks, swans and geese migrate between summer and winter seasons, most of the ducks in Australia do not regularly leave the continent with two exceptions: the wandering whistling ducks and the magpie goose (8). The latter is susceptible to experimental infection with H5N1 HPAIV resulting in clinical disease and mortality (D. Middleton, AAHL, personal communication). Munster and Fouchier (4) indicated that ducks

infected with H5N2 HPAIV were able to migrate more than 650 km and it therefore conceivable that infected magpie geese can introduce H5N1 HPAIV into Australia. It has to be realized that many other species of free-living birds can become infected and die as a consequence. It is not known how soon after infection these birds become clinically ill or if they do not become sick if they can transmit the virus.

Fig 1. Outbreaks of H5N1 HPAI during the period of 29-10-2009 to 29-4-2010. (http://www.fao.org/avianflu/en/maps.html, Accessed May10, 2010)



Munster and Fouchier (4) reviewed the ecology of AIV and offered some interesting insights into the differences between the Anseriformes and the Charadriiformes as ecological reservoirs for AIV. The Charadriiformes, and especially the gulls and terns, are the natural hosts for the H13 and H16 subtypes, while the Anseriformes are the natural host for the other 14 subtypes. However, this separation is not absolute and AIV subtypes other than H13 and H16 viruses have been isolated from sandpipers in Australia. For example, Hurt et al (2) obtained H4N8 isolates from red-necked stints and H11N9 isolates from sharp-tailed sandpipers, but these viruses were low pathogenic. It is currently unknown how widespread infection with subtypes other than H13 and H16 is in Charadriiformes. Experimental infection of ducks with gull viruses indicated that ducks are not highly susceptible to these viruses, which may be related to subtle differences in the HA receptors between these viruses (reviewed in 4). However it is unknown how susceptible Charadriiformes are to infection with any of the 14 subtypes associated with ducks. It is also not known how often these birds co-mingle on the breeding grounds with dabbling ducks in Asia prior to migrating to Australia. In addition it is not known how these viruses and especially H5N1 HPAIV would affect the ability of long-range migrants to reach Australia. Answers to these questions are essential to address the question how easy H5N1 HPAIV can be introduced in Australia by free-living birds. On the other hand introduction of the virus by fishing boats from Indonesia may constitute a far greater risk to native duck populations.

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Avian Influenza Vaccination – Preparing For an Outbreak

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Introduction

The Australian government has been enhancing preparedness activities for avian influenza since widespread outbreaks of H5N1 highly pathogenic avian influenza (HPAI) were reported in Asia from 2003. These preparedness activities have included re-evaluation of the role of vaccination in control of avian influenza (AI) of the H5 and H7 subtypes.

Australia has had five outbreaks of HPAI in the past. Each of these outbreaks was eradicated by stamping out (destruction of infected flocks) without vaccination. Australia's preferred approach to an outbreak of HPAI or low pathogenicity AI (H5 or H7) is to eradicate the virus without vaccination. However, vaccination would be considered in the event of a large, rapidly spreading outbreak of either HPAI or LPAI (H5 or H7) in Australia. If a decision is made to vaccinate, the strategic priority will be to stop the spread of the outbreak and to protect rare, endangered, and valuable birds.

Recognising the growing importance of vaccination in controlling emergency animal diseases, the Animal Health Committee formed a national AI vaccine expert (NAIVE) group to provide advice on vaccination, refine vaccination policy and keep a watching brief on vaccine developments. NAIVE is coordinated by the Australian Government Department of Agriculture, Fisheries and Forestry, and group members include consultants to the poultry industry with particular expertise in vaccines and vaccination, as well as members from state and Commonwealth governments.

Regulatory approval of AI vaccines

The Australian Chief Veterinary Officer currently holds import permits for emergency use for four inactivated AI vaccines – Intervet Nobilis Influenza H5 (H5N2), Intervet Nobilis Influenza H7N1, Pfizer (Fort Dodge) Poulvac Flufend i AI H5N9 and Pfizer Poulvac Flufend i AI H7N1. All four vaccines are also registered with the APVMA for emergency use in Australia. If these vaccines are ordered in response to an outbreak of AI (H5 or H7), they may only be released from a quarantine-approved premises with the written authorisation of the Chief Veterinary Officer following a decision by the Consultative Committee for Emergency Animal Diseases (CCEAD) to use vaccination as part of an emergency response to an outbreak.

Process of selection of AI vaccines

Vaccines suitable for use in Australia were selected based on a number of considerations. The preference was for a vaccine that was registered or approved in a reputable country (for example, the United States or Europe), for which safety and efficacy data were available, as well as evidence of good manufacturing processes. Inactivated vaccines generally result in a stronger immune response than the genetically modified vaccines available at the time. Potential delays in getting regulatory approval for genetically modified vaccines for food-producing animals, as well as issues with consumer acceptance, were taken into account during the selection of suitable vaccines.

Decision support tool for AI vaccination

A decision-support tool has been developed to assist CCEAD to determine if vaccination should be used as part of an eradication program in the event of an outbreak of AI due to an H5 or H7 subtype. The decision support tool takes into account the location of the outbreak (e.g. in an area of low or high poultry density), likelihood of spread, availability of vaccine and resources, public reaction to a stamping out policy and so on. The decision support tool has been used in desk-top

simulations of outbreak scenarios and is currently available for comment by industry.

National operating procedures for AI vaccination

A set of draft national operating procedures (NOPs) has been developed by the NAIVE group as a basis for discussion with industry. Once agreed between industry and Animal Health Committee, this document will be linked to AUSVETPLAN and will form part of the nationally agreed response to an outbreak. The document will also be used to develop detailed standard operating procedures for entry and exit procedures from farms, biosecurity arrangements for vaccinators, pre-vaccination testing and post-vaccination monitoring of flocks, and so on.

The draft NOPs describe principles for vaccinating commercial and non-commercial poultry within an area subject to an emergency response plan. The document covers who can authorise vaccination; the importation and distribution of AI vaccine and under what circumstances it will be released from quarantine; pre-vaccination testing of flocks (to ensure the flock to be vaccinated is not already infected); movement controls for vaccinated flocks and their products; monitoring and testing of vaccinated birds; testing to prove freedom once the outbreak is eradicated; inventory control and exit strategy from the vaccine campaign.

Because of the nature of the vaccine (requiring administration to individual birds) and the length of time required for development of immunity, it is likely that longer-lived flocks, such as breeders, layers and turkeys, would be targeted for vaccination during an emergency response. It is possible to vaccinate short-lived birds, however, if such flocks are at risk from spread of AI virus, it may be logistically more practical to depopulate or send them for early processing.

Vaccination carried out as part of the emergency response would funded from cost-sharing arrangements.

Vaccination of flocks outside the emergency response

The poultry and zoo industries have expressed interest in having access to vaccine to protect valuable or rare birds from avian influenza in the event of an outbreak occurring in Australia. These flocks may not necessarily be situated in an area subject to an emergency response, or, if they are within such an area, may not be otherwise considered for vaccination as part of the response plan.

A draft document, currently with industry groups for comment, takes into account proposals to vaccinate flocks or birds 'outside' the emergency response plan. Applications to vaccinate such flocks would be evaluated on a case-by-case basis by CCEAD, taking into account national and state interests, vaccine availability, risk of exposure to AI, and other factors. Flocks vaccinated outside an emergency response plan would be vaccinated at the expense of their owners. They would be subject to movement controls, may be required to accommodate unvaccinated sentinel birds, and would undergo monitoring and testing, also at the owners' expense. The frequency of monitoring would be risk-based and depend on individual circumstances, including flock size, proposed movements of products or birds, biosecurity level and so on.

Vaccination of zoo birds

A draft document on vaccinating zoo collections, captive rare breeds and endangered species is currently under consideration by the zoo industry. Procedures for application, vaccination and testing of the zoo birds are similar to those described for vaccination outside the emergency response, and the cost of vaccination and testing would be borne by the owners of the bird collection.

Administration of vaccines

The NAIVE group has had discussions with commercial poultry vaccination companies about the practicalities of administering AI vaccines to uninfected flocks during an emergency response. While some larger poultry companies are able to supply their own vaccination staff, NAIVE recognises that the use of commercial vaccination crews in an emergency could enhance the efficiency of vaccination, welfare of birds being vaccinated, the number of birds that can be vaccinated, as well as providing access to trained poultry handlers, vaccinators and other contacts within the poultry industry. Some commercial vaccination crews have agreed to provide input during the development of standard operating procedures for AI vaccination, and to supply a core of staff to assist in training of vaccinators

in an emergency. NAIVE also recognises the need for such companies to maintain business continuity during an outbreak by reserving 'clean' crews to service their usual clients.

Vaccine supply

At the time of selection of the four vaccines registered for emergency use in Australia, each of the vaccines was maintained in a ready-to-use form by the vaccine manufacturers. However, both Intervet and Pfizer have recently advised the Office of the Chief Veterinary Officer that they no longer stock ready-to-use H7N1 vaccines, and that the time to manufacture and safety testing of a batch of vaccine could be 4-6 months from the date of order. Therefore, despite careful vaccine selection and regulatory approval, Australia does not currently have access to a ready supply of vaccine against an H7 AI virus. The next step in our vaccination preparedness plans, therefore, is to revisit the subject of contingency vaccine supply options with industry, state governments and vaccine manufacturers.

The National Newcastle Disease Management Plan - 2002 to 2012

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SUMMARY.

Australia developed a National ND Management Plan (NNDMP) (the "Plan") from 2003 to attempt to eradicate and prevent the re-emergence of precursor and virulent ND virus (vNDV). Because of the potential for vNDV re-emergence and a decision that a test and slaughter approach would not be successful, a vaccination strategy using the live lentogenic strain V4 and inactivated ND vaccines was developed to out-compete precursor viruses that have a closely related sequence to that of the vNDV. The extent to which vaccination can prevent the spread of precursor virus is paramount to the success of the program. The Plan has now been revised three times. This paper describes the origins of the Plan and the progress that the Plan has made over the past eight years in preventing ND and looks ahead towards the end of the current Plan in 2012 when the possibility of a cessation of ND vaccination could be realised.

INTRODUCTION

Australia would appear to have experienced a rather unique event in terms of the mutation of vNDV from an endemic lentogenic strain. The factors that encouraged precursor and vNDVs to emerge are not well understood, although flock immunosuppression due to concurrent MDV, IBDV or CAV infection has been suggested to alter selection pressure during virus propagation through the flock, assisting evolution to virulence. Alternatively, it has been suggested that vNDV may have emerged slowly in accordance with the quasi-species concept with evolutionary selection pressures on a heterogeneous population of NDVs causing those sub-populations to emerge that are best adapted to the changing poultry growing environment. Because of the potential for vNDV re-emergence, a vaccination strategy was developed that aimed to out-compete precursor viruses that have a fusion protein gene sequence similar to that of the virulent virus.

A Government Industry Technical Working Group developed extensive standard operating procedures (SOPs) during the period 1999-2000 to effect ND control in NSW when Australia had no national policy to control vNDV arising out of an endemic strain. Reluctance by government to implement compulsory vaccination was heavily criticised at the time. However, a national survey in 2000 indicated that vNDV and progenitor viruses were not widespread. Further outbreaks from vNDV in Victoria and NSW in 2002 forced the issue and out of an Emergency Animal Disease response Agreement (EADRA), a request was made to Animal Health Australia (AHA) to develop a management plan including compulsory vaccination. AHA appointed a Steering Committee to develop the Plan.

THE NATIONAL ND MANAGEMENT PLAN

In 2003, the Steering Committee of the NDNMG recommended an integrated risk-managed approach to ND prevention. The integrated risk management approach was aimed to deliver the following goals:

a) Minimise the risk of ND outbreaks from Australian-origin virulent viruses
b) Protect the status of non-infected flocks and regions; and
c) Reduce the social, economic and trade impact of ND at farm, regional and national levels.

The operational projects to deliver these goals comprised:

1. A control project involving biosecurity plans, strategic vaccination and other agreed standard operating procedures (SOP).
2. A national surveillance project to detect the presence of precursor and virulent ND viruses.

3. A communication project to promote awareness in the poultry industry.
4. A research and development project to support the Plan.
5. A management and evaluation project to provide co-ordination and review implementation of the Plan.

In the 2005-2007 Plan, more specific targets were set including:

- the application of poultry industry biosecurity plans
- the strategic application and monitoring of vaccination
- nationally coordinated surveillance to identify the location of precursor viruses
- communication and awareness to promote early detection of ND
- a review of the implementation of the Plan.

The 2008-2012 Plan included the same components of the previous Plan together with some reduction in compulsory vaccination in states considered to be of lower risk. It also included a risk-based exit strategy that will potentially minimise or eliminate vaccination as a requirement to prevent outbreaks of Australian-origin ND. The risk-based exit strategy includes an assessment of existing and planned surveillance data to support the current program and proposed changes into the future.

REGULATORY VACCINATION REQUIREMENTS

ND vaccination was introduced in NSW in 1999 became compulsory around Sydney in 2002 and throughout NSW in 2003. However, it was not until 2005 that all Australian states required commercial poultry to follow mandatory vaccination programs as described in a set of SOPs developed by the NDMG. The SOP for ND vaccination of broiler chickens recommended that chickens be preferably vaccinated with live V4 strain ND vaccine at 7-14 days of age. However, the SOP also allowed farmers the option of vaccinating at day-old provided they can show evidence of equivalence to the preferred program. The latter program retains control of ND vaccination in hands of the hatchery where it has been claimed that improved uniformity and lower cost can be achieved than through administration by drinking water undertaken by broiler growers. Adequacy of the response to vaccination is defined as the mean haemagglutination inhibition (HI) titre of the flock being at least log 2^{3} with at least 66% of the individual samples reaching a HI titre of 2^{3} by 35 days of age. Commercial layer and breeding flocks were to be vaccinated with inactivated ND vaccine after initial priming with live V4 vaccine.

ISSUES ARISING DURING THE IMPLEMENTATION PHASES

A review of the Plan in 2006 by Andrew Turner Consulting concluded that the Plan had achieved a major goal in preventing outbreaks of ND since 2002. The review also identified a number of deficiencies in the Plan. These included dissension within the industry and government agencies as to the direction of the Plan, the choice of V4 vaccine or its method of administration providing sub-optimal protection against precursor viruses especially in broiler chickens, inadequate data on vaccine usage, the lack of a targeted surveillance program, a need for an improved and audited biosecurity program and an appropriate exit strategy.

The Steering Committee agreed that there was a need for a risk analysis to modify the Plan and that there were major discrepancies in the usage of vaccine and the numbers of birds to be vaccinated across Australia. However, some recent studies by Arzey and Arzey (2008) refuted the concerns about the efficacy of V4 vaccine in broiler chickens. The Committee agreed that recommendations from the review would be considered in the Plan beyond 2007.

In 2008, the Steering Committee embarked on a new Plan for 2008-2102 that included a three-tier risk-based assessment of the vaccination programs required for states perceived to be at a lower risk than NSW and Victoria. It was proposed (at level 1) that compulsory vaccination of broilers be removed in Tasmania and Western Australia (WA). This would also include voluntary use of killed ND vaccination of laying flocks. At level 2, in South Australia and Queensland, compulsory use of killed ND vaccine in layers would be removed but that regular use of live vaccine would be a compulsory alternative coupled with monitoring. At level 3 in NSW and Victoria the vaccination SOPs would not change. These changes were supported by a risk assessment and were supported by a

proposed surveillance plan. The AHC endorsed the new Plan after a risk assessment for the proposed changes in SA and Queensland indicated the risk of vNDV or progenitor virus existing in those states was of low risk. The Risk Assessment also indicated that the overall risk in other jurisdictions including NSW and Victoria was very low. An evaluation of a surveillance program remained incomplete until testing was undertaken in WA and Tasmania. Procedures were put in place for an improved evaluation of compliance to the vaccination SOPs. The Plan is due for mid-term review later in 2010 where further consideration will be given to an exit strategy, the surveillance plan report outcomes, vaccine usage compliance (only for layers) and the inclusion of endemic NDV in the EADRA.

FUTURE DIRECTIONS

Generally, it was felt that the success of the risk-based strategy proposed in the Plan would depend upon a number of factors, some of which are related to the biology of the virus, and others dependent upon the enthusiasm with which the poultry industry and government face the problem. Shifts in virus population dynamics from ND vaccination have previously been proposed and the removal of vaccination could unmask progenitor or vNDVs that have been shown to exist within quasi-species populations. We may be fortunate that selection pressures that existed in the 1990s now no longer exist. However, a precautionary approach has been taken in the Plan in the likelihood that the risks are greater than can be calculated. Failure to communicate with the other sectors of the poultry industry on the need to vaccinate and maintain high levels of biosecurity could allow precursor virus to persist and prevent total eradication although history is perhaps against this occurring.

REFERENCES

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ACKNOWLEDGMENT

The comments by Dr George Arzey on the draft of this paper are greatly appreciated.

Report on the Activities of the International Poultry Council

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Introduction

The International Poultry Council (IPC) was formed in 2006, with the intention of bringing together poultry industry leaders from around the world to discuss and address issues of trade, science, and to improve relations among poultry producing nations.

The stated objectives of the IPC are to strengthen communication between the industries of different countries, to develop and recommend policies affecting the poultry industries of all countries, and to promote a common global understanding of and confidence in poultry products as the preferred source of meat protein.

The IPC meets twice a year. Outside of meetings, activities of the IPC are coordinated by its Executive Committee and convenors of several working parties.

ACMF's Participation in the IPC

The ACMF joined the IPC in 2007 and has attended five of its meetings, the most recent being held in Paris on 14th – 16th April 2010. The ACMF in fact hosted the previous IPC meeting in Sydney in October 2009. Vivien Kite represents the ACMF on the IPC, and was elected to the Executive Committee of IPC at its Sydney 2009 meeting. She also convenes the IPC's informal working groups on animal welfare and has responsibility for leading IPC activities relevant to OIE's activities in the food safety area.

Membership

The IPC now has 23 member countries, including the world's largest producers, users and traders in chicken meat, including the four largest producers (USA, China, Brazil, EU, Mexico), the six largest consumers (USA, China, EU, Brazil, Mexico and Russia), and the eight largest exporters (Brazil, USA, EU, Thailand, China, Argentina, Canada, Chile) of chicken meat in the world.

Associate membership of the IPC now includes an increasing number of major individual suppliers to and customers of the industry, including Cobb-Vantress, Aviagen, Cargill, Yum! and McDonalds, as well as several major producers, such as Tyson Foods.

Delegates from 19 countries attended the recent Paris 2010 meeting, as did representatives of the majority of associate members.

Recognition of IPC by Intergovernmental Bodies

During the Paris 2010 meeting, a memorandum of understanding was signed between the IPC and the Food and Agriculture Organization of the U.N. (FAO), which officially recognises the IPC as the international organisation with which the FAO will confer when addressing issues relevant to the poultry industry. This is the third international policy and standards setting organisation which has officially recognised IPC. In 2008 the IPC gained the recognition of the OIE. The IPC has also been officially recognised by the Codex Alimentarius Commission. Recognition by these organisations generally gives IPC observer status in their consideration of issues and the ability to provide greater input into standards or policies developed by them. It also provides expanded opportunities for the world's poultry industries to contribute to the work of these organisations' various working and expert groups. For example, the IPC was invited to participate in two OIE *ad hoc* working groups over the past year - one on private standards in animal health and animal welfare, and the other to develop a standard on Animal Welfare and Chicken Production Systems. Dr Thomas Janning (Germany) represented IPC on both of these working groups.

The IPC is also seeking recognition from the World Health Organisation.

IPC Activities and International Developments

OIE

The first draft of a new OIE Terrestrial Animal Health Code chapter on Animal Welfare and Chicken Broiler Production was developed by the ad hoc working group referred to above at its first meeting in June 2009. IPC member country comments on this first draft are currently being compiled so as to provide input into the ad hoc working group's next meeting in June 2010. Where consensus across the membership cannot be achieved, IPC input into the second draft of the document will be worked through with members at the IPC's next meeting in October 2010. Following consideration of comments from member countries and supporting organisations (such as IPC), this new chapter may be considered for adoption in May 2011.

There are several existing Chapters of the OIE's Terrestrial Animal Health Code that are relevant to poultry welfare (eg Chapter 7.3 Transport of Animals by Land; Chapter 7.5 Slaughter of Animals; Chapter 7.6 Killing of Animals for Disease Control Purposes). As these periodically come up for review, Australian industry is in a strong position to have input into them through our IPC involvement, as well as through our own national processes (led by DAFF).

A revision of two existing OIE Terrestrial Animal Health Code chapters relevant to Salmonella control in poultry (to be consolidated into a single draft chapter - Chapter 6.5 Prevention, Detection and Control of Salmonella in Poultry) will be put up for adoption at the General Session of the OIE at its meeting in June 2010. While there is no opportunity for input by IPC into this chapter at this late stage, Chapter 6.4 of the OIE Code (Hygiene and Biosecurity Procedures in Poultry Production) is currently being reviewed and IPC will be given an opportunity to provide input into the revised chapter following its public release later in 2010.

The OIE has published guidelines to support the practical application of the concept of compartmentalisation (TAHC Chapter 4.4 Application of Compartmentalisation) and is involved in two pilot projects on compartmentalisation in the poultry sector. These two projects (one for AI and the other for both AI and ND) are being undertaken by the two countries concerned (Brazil and Thailand), with OIE providing technical advice. The reports of these two projects will be confidential to the countries concerned. However, in their country's report to the IPC, the delegation from Thailand indicated that technical feedback received from the OIE so far has suggested that they still had some way to go in terms of implementation of HACCP principles at the (meat chicken) farm, and also the extension of the biosecurity management system and HACCP arrangements to the breeding farms supplying the meat farms in the compartment.

Codex

In the course of 2009, Codex Alimentarius released a report on Salmonella and Campylobacter in Chicken Meat. In its assessment of the Codex draft 'Guidelines for control of Campylobacter and Salmonella spp. in chicken meat' the expert committee that undertook the scientific review of the draft guidelines questioned the value of chlorination as a means of controlling these pathogens at key points in processing. The status of chlorine for disinfection of carcasses in processing remains unresolved. Al Yancy (USA) will be representing IPC in any further work on these Codex draft Guidelines.

GMOs

As is the case in Australia, products derived from animals fed GM feed ingredients do not have to be labelled as GM in the EU. However, this year, the EU will be looking into extending the labelling requirements to end-products which have come from animals fed GM feed ingredients. If endorsed, this will apply to imported animal food products as well as locally produced products.

Given the strongly held views of some EU poultry producing industries that they should maintain a 'non-GM' policy, it is unlikely that IPC will be able to develop or progress a common policy and communications activities with respect to the GM issue.

Poultry Meat Promotion

One of the IPC's objectives is to promote a common global understanding of and confidence in poultry products as the preferred source of meat protein. While no major initiatives in this area have yet been undertaken, the IPC will be initiating an annual marketing award, commencing in April 2011, one aim of which will be to share communications, educational and promotional materials and ideas which may be extended globally, or which may simply be adopted by other member counties.

World Trade and Production Trends and Policy Developments

One of the major benefits of ACMF's involvement with the IPC is that it enables the Australian industry to gain a better understanding of trends in world production and trade in poultry products and to obtain insights into evolving issues and policy developments around the world which have the potential to impact on our own industry in the future. The member country reports which are delivered at every second IPC meeting are an invaluable source of this information, as are the informal discussions held with delegates at all meetings. As an example, the major concerns of the EU poultry industries often revolve around impending or recent changes in EU legislation. At the moment, there is considerable concern regarding the implementation of EU Regulation 2160/2003 – Annex II, which requires, by 12 December 2010, the absence of *Salmonella* in 25g of fresh chicken and turkey meat. It is not clear yet how this regulation will be applied (for example, whether it will only be applied to certain critical serovars such as Enteritidis and Typhimurium), and the industry is hoping for clarification of this in the near future.

The implementation of a recent EU regulation on electrical stunning criteria (which sets defined currents to be applied at various frequencies) has had serious impacts on product quality, which has led to a major search for alternative approaches, with some promising alternatives being develop in the Netherlands in particular.

Further details on the markets, statistics and regulatory environment of the poultry industries in each of the member countries are contained in the country reports from the IPC's most recent meeting (Paris 1010) which are held by the ACMF.

Conclusions

The IPC is a young organisation, but one which is growing in membership and is increasingly being recognised as the peak international body representing the interests of the poultry meat industries globally. Participation in the IPC and its meetings has provided us with new opportunities to understand developments in the poultry industry globally, and to input into international standards and policy setting processes. The ACMF has recently committed to retain its membership of the IPC, and to strengthen its participation by attendance at all IPC general session meetings, at least until the end of 2011.

Poultry Disease and Control in Overseas Countries – Relevance to Australia

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Introduction

Australia is fortunate in not having some poultry diseases/infections that occur in other countries. Unlike many overseas countries, Australia being an isolated island continent, has no land borders with other countries, but Australia is on the migratory path of some wild bird species including waterfowl. Australia has strict government quarantine regulations and import requirements. Import of breeder hatching eggs has been permitted for some years, but only through quarantine stations with rigorous biosecurity precautions and disease testing. Import of inactivated vaccines is permitted. Imported live vaccines are produced from Australian SPF eggs, except by special authorisation which is currently occurring. Australia has very strict import requirements for chicken meat, table eggs and other poultry products.

Economically Important Poultry Diseases/Infections in Australia

Viral Diseases

- Infectious Laryngotracheitis, Infectious Bronchitis, Avian Encephalomyelitis, Chicken Anaemia, Inclusion Body Hepatitis, Fowl Pox, Marek's Disease, Leucosis/ Reticuloendotheliosis, Egg Drop Syndrome, Runting Stunting Syndromes, Proventriculitis, Intermediate+ Infectious Bursal Disease Infections, Turkey Haemorrhagic Enteritis

Bacterial Diseases

- Necrotic Enteritis, Colibacillosis, Femoral Head Necrosis, Paratyphoid Salmonellosis, Fowl Cholera, Infectious Coryza, Enterococcus spp, Mycoplasma spp, Campylobacter Infections, Duck Infectious Serositis, Erysipelas, Avian Chlamydiosis

Protozoan/Fungal Diseases

- Coccidiosis, Blackhead, Aspergillosis, Mycotoxicosis

Internal/External Parasites

- Roundworms, Tapeworms, Mites, Lice

Economically Important Poultry Diseases/Infections Not In Australia

Viral Diseases

- Gumboro Disease, Variant Infectious Bronchitis, Avian Pneumovirus Infections (Turkey Rhino-tracheitis, Swollen Head Syndrome of Chickens), Virulent Newcastle Disease, Avian Influenza, Duck Hepatitis, Duck Viral Enteritis

Bacterial Diseases

- Fowl Typhoid (*Salmonella gallinarum*), Virulent *Salmonella enteritidis* infections, *Ornithobacterium* Infections, Infectious Coryza Type B

Examples of Vaccination Programs in Overseas Countries

Breeders

Age	Vaccination	Method
Hatchery	Marek's (Rispens)	Injection
5 days	Inactivated ND + Live IB	Injection + Eyedrop
6 days	Live Salmonella	Drinking Water
7 days	Coccidiosis	Drinking Water
9 days	Live Reovirus	Injection
10 days	Variant IB	Spray
14&20 days	Live IBD	Drinking Water
21 days	Variant IB + Live ND	Spray
38 days	Live Salmonella; Live MG&MS	Drinking Water; Eyedrop
49 days	Live IB + Live ND	Spray
70 days	Live Avian Pneumovirus	Eye Drop
80 days	ILT; Inactivated Fowl Cholera	Eyedrop; Injection
84 days	Inactivated SE+ST; Live AE+TRT; Inactivated Fowl Cholera	Injection
90 days	Live IB + Live ND	Spray
119 days	Inactivated ND+IB+IBD+TRT; Inactivated Reovirus; Inactivated Infectious Coryza; Inactivated Fowl Cholera	Injection
35, 43& 51 wks	Live IB (Variant at 43 wks); Live ND	

Broilers

Age	Vaccination	Method
In Ovo or Day Old - Hatchery	Marek's (HVT)	Injection
10 days	Live IB (Variant) + Live ND	DW
12&18 days	Live IBD	DW
20 days	Live ND	DW or Spray

Gumboro Disease Caused By Very Virulent Infectious Bursal Disease (IBD) Virus

Very virulent IBD occurs in all countries except Australia and NZ. Mortality of up to 20% can occur in chickens over 3 weeks of age. Very virulent IBD virus also causes immunosuppression, as do other less virulent classical and variant IBD viruses. Control is by vaccination of broilers either once or twice, usually with intermediate+ vaccines. Maternal antibody (MAB) levels of broilers have to be taken into consideration to achieve effective vaccination. Intermediate+ vaccines can cause bursal damage if young broilers have low levels of MAB while high MAB interfere with effective vaccination. The Deventer formula is used in Europe and western Asia to estimate the age of vaccination and whether one or two vaccinations are required. The MAB titres of 5-day old broilers from various breeding flocks are inserted into the Deventer calculator to decide when to vaccinate. Vector vaccines and antigen-antibody combination vaccines are available but are not widely used for control of Very Virulent IBD virus. The economic cost of the disease is due to mortality, immuno-suppression, overall decreased productive performance and vaccination costs.

Variant Infectious Bronchitis (IB)

There is a number of IB virus variants reported around the world, often requiring new vaccines for control. The QX variant in Asia and Europe is one of the most economically important variant IB viruses. This IB virus variant causes a severe disease in broilers, breeders and layers. Infection in layers or breeders with QX variant in the first few weeks of life causes “false layers” with oviduct abnormalities. Affected birds do not lay eggs. The harmful affects of this variant is only discovered when the flock comes into lay and even then affected hens can’t be identified for culling as they appear to be normal birds. A special vaccine has been developed for prevention – but of course this adds cost to health programs.

Virulent Newcastle Disease (ND)

ND is still one of the most important economic diseases of poultry worldwide, despite vaccination being the main control measure. Vaccine reactions and inadequate control of virulent virus can contribute to late respiratory disease, particularly if there is immuno-suppression due to IBD virus. Virulent ND occurred in Australia in commercial poultry in NSW and Victoria in 1998-2000 and was eradicated. The Australian V4 virus vaccine was used to assist eradication of locally-derived virulent ND in Australia. Will V4 vaccine be sufficient to control other strains of ND virus should these gain access to Australia and become endemic?

Avian Influenza (Ai)

Avian Influenza has become an important zoonotic disease of poultry in recent years, particularly because of the spread of H5N1 AI virus though Asia and Europe, but also because H7 AI virus also caused human disease. Australia has had five outbreaks of H7 AI since 1976, all of which have been eradicated. Surveillance test evidence of H5 AI virus in wild birds in Australia in recent years is of epidemiological significance. Biosecurity procedures, particularly related to water fowl and their droppings, need to be actively managed. Vaccines have been approved for use in eradication programs in Australia as part of the national AI AUSVETPLAN. In addition to the poultry disease and human health consequences, outbreaks of AI can have devastating effects on poultry sales, at least in the short term.

Salmonella enteritidis (Se)

SE infection causes poultry disease, but more importantly is the major cause of salmonella food-poisoning of humans in many countries. SE PT26 occurs sporadically in Queensland, but has not caused disease in poultry or food-poisoning outbreaks. The cost of endemic SE infection is due to lack of confidence in the safety of poultry products, particularly eggs, which can result in decreased sales and ongoing costs of control in poultry, mainly vaccination of breeders but also medication of broilers.

Pneumovirus Infections

Avian Metapneumonovirus infections occur in most countries except Australia and NZ causing Turkey Rhinotracheitis (TRT) and Swollen Head Syndrome (SHS) in chickens which are also called avian rhinotracheitis (ART). A severe respiratory disease and egg production drops in turkeys and chickens result. The respiratory disease in chickens can occur subsequent to infection with other respiratory viruses and can be complicated by bacteria such as E. coli. Control is by vaccination in

breeders and turkeys, but vaccines are not often used in chickens because of the cost. This virus exacerbates the severity of poultry respiratory disease due to other causes with consequent mortality/morbidity and egg production losses which require costly vaccination programs to prevent.

Summary

So despite all precautions, Australia does have many economically important diseases which are usually well controlled but at a cost in vaccination, medication and biosecurity/ hygiene measures. A number of economically important diseases that occur in overseas countries do not occur in Australia. The occurrence of these diseases in Australia would increase the costs of poultry production in mortality, decreased productive performance and control measures and in some cases increase the risk to human health.

An Update of Recent Infectious Laryngotracheitis Virus (ILTV) Strains Identified in Australia

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Summary

In recent times, numerous outbreaks of Infectious Laryngotracheitis (ILT) have occurred in poultry in Australia, particularly in Victoria (VIC) and New South Wales (NSW). The objectives of this study were to identify the viral strains involved in the outbreaks over the last twelve months and to determine epidemiological relationships of isolated viruses.

A combination of polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses of ILTV genes was applied to identify genetic differences in the field and vaccine viruses. In previous studies conducted in our laboratory, these procedures had demonstrated nine RFLP classes (1 to 9) of Australian ILTV strains. Both Fort Dodge vaccine strains, A20 and SA2, belong to class 1 ILTV, whereas Class 7 is the Nobilis (Serva strain) ILT vaccine (Intervet Pty Ltd). The remaining classes of ILTV are field strains that have been identified and characterised in chronological order, as they were discovered.

Class 9 was the most prevalent strain identified in the current study, with 32 cases from VIC and NSW in the past year. This strain was not found in any submissions from Queensland (QLD), South Australia (SA) or Western Australia (WA). In VIC, class 9 has displaced the previously endemic class 2 virus and spread from one side of Port Phillip Bay to the other. The series of outbreaks leading to this event suggests a lapse in biosecurity measures employed by staff and contractors travelling between farms.

Class 2 was found in both Queensland and Victoria, but time of onset and previous records of existence of class 2 in these regions indicate that there is no significant link between the outbreaks. Class 3 was found only in game bird populations in NSW, QLD and WA and suggests a link with game bird transport between these states.

In Australia, Infectious laryngotracheitis (ILT) causes substantial economic losses to both layer and broiler production companies. It is a highly contagious respiratory illness affecting gallinaceous birds and is associated with acute respiratory distress and reduced egg production (5). The virus is a member of the *Herpesviridiae* family, and like other herpes viruses can induce a state of latency in carrier birds (2,7,8). ILT can spread between farms in close vicinity to each other by fomites and/or aerosols (9).

Embryo propagated vaccine ILTVs have previously been found accountable for field outbreaks in the US (3,11) so the ability to differentiate between 'wild-type' and vaccine strains of ILTV is of high importance to the Australian poultry industry.

A range of studies have focused on the differentiation of ILTV genotypes across various countries by using restriction fragment length polymorphism (RFLP) patterns from endonuclease-digested PCR products (1,4,6) and this has recently become the most widely-accepted method for genotyping ILTV isolates. Some research has been based on the differentiation of vaccine and field isolates using these patterns (1,4). More recently, a combination of RFLP patterns for a number of ILTV genes has been used for classification of ILTVs in Australia (10) and overseas (11). Application of this methodology on ILTVs isolated from different locations in Australia has resulted in identification of 9 different ILTV classes, with the SA2 and A20 vaccine strains classified as class 1 (10) and the Nobilis

vaccine classified as class 7. The main purpose of the current study was to use this methodology to identify the ILTV classes responsible for the recent outbreaks of ILTV in Australia, to establish if there are any links between the outbreaks occurring in different states and to investigate if the vaccine strains may be linked to these outbreaks.

Materials and Methods

Viruses. The commercial ILTV vaccine strains SA2, A20 and Serva, along with ILTV field isolates collected over the last twelve months were used in this study (Table 1). All field isolates were isolated from the upper respiratory tract and/or conjunctiva of infected birds during outbreaks of ILT in Australia. All but three submissions for this study were made from commercial layer and broiler farms experiencing mortalities associated with ILT. Three submissions were from flocks of game birds (Table 2). A vaccine strain associated solely with morbidity, but not significant mortality, was not included in this study. A total number of 73 ILTV isolates were examined in this study, of which 49 were from VIC, 12 were from QLD, 9 were from NSW, 2 were from SA and 1 was from WA.

Extraction of viral DNA. DNA was extracted from infected CEK cell supernatant, commercial vaccines, or directly from swabs taken from infected trachea using a method described previously (10). Where insufficient amplification was achieved in PCR, virus isolates were propagated on chicken embryo kidney (CEK) cells using standard techniques (12). Scrapings of affected trachea and/or conjunctiva were diluted in cell culture medium and inoculated onto CEK cells. Once infected, CEK cells were frozen at -80ºC, thawed, centrifuged at 500 × g for 5 min, the supernatant removed and used for DNA extraction. The extracted DNA samples were used immediately or stored at -20ºC before use in the PCR.

Polymerase chain reactions. Primers for the genes thymidine kinase (TK), infected cell protein 4 (ICP4) and infected cell protein 18.5 (ICP18.5) were used in PCR as described previously (10) with some modifications. The ICP4 and ICP18.5 PCRs were carried out by subjecting the reactions to 94^{o}C for one minute followed by 35 cycles of 94^{o}C for 15 sec, 64^{o}C for 45 sec and 68^{o}C for 5.5 minutes, and a final extension of 68^{o}C for 10 min. All PCRs were performed using Platinum Taq DNA polymerase high fidelity (Invitrogen, Carlsbad, CA). Control tubes containing distilled H2O, instead of extracted DNA were included as the negative control in all series of PCR. The PCR products were separated by electrophoresis in 1% agarose gels, stained with GelRed (Biotium, Hayward, CA), and exposed to ultraviolet light for visualization using the Kodak Gel Logic 1500 Imaging System with Kodak Molecular Imaging Software (Version 4.0.5, 2005) system.

RFLP and staining. Ten µl volumes of PCR products were digested separately with the restriction endonucleases at 37ºC for 2 hr as described previously (10). The restriction endonuclease enzyme *Msp*I was used for the TK gene products and *Hae*III for the ICP4 and ICP18.5 PCR products. After digestion, the resultant DNA fragments were separated on a 15% polyacrylamide gel. Restriction DNA fragments on polyacrylamide gels were visualized by staining with GelRed (Biotium, Hayward, CA), and subjected to digital imaging using the Kodak Gel Logic 1500 Imaging System with Kodak Molecular Imaging Software (Version 4.0.5, 2005) system.

Results and Discussion

A total number of 73 samples from VIC, QLD, NSW, SA and WA were referred to our laboratory for ILTV detection and strain identification in the last twelve months. These consisted of 49 submissions from VIC, 12 from QLD, 9 from NSW, 2 from SA and 1 from WA.

Three cases were negative for ILTV and thirteen cases could not be typed due to virus degradation in the tissue submitted. Twelve cases were excluded, as they were associated with no or minor mortality due to a vaccine reaction (as confirmed by a detection of class 1 or 7 genotype only). Of the two samples submitted from SA, one could not be typed due to virus degradation and the other contained a class 7 ILTV from a game bird and was therefore excluded. The remaining 45 submissions made over the last year were from VIC, QLD, NSW and WA and are presented in table 2.

From outbreaks associated with mortality, ILTV classes 2, 3, 8 and 9 were detected. The viruses were detected from a range of chicken flocks, including commercial broilers and layers and game birds.

The majority of the viruses were identified as class 9 ILTV, most of which were submitted form Victorian farms. The first case of a class 9 ILTV in Victoria detected by our laboratory was in November 2009, submitted from a layer farm on the outskirts of the Geelong region. The Intervet Nobilis vaccine was in use at this farm (personal communication with the company veterinarian). The next few months saw the class 9 virus appear in Melbourne's outer western suburbs, central VIC, and then the Gippsland region. A total of 29 cases from VIC over the last year resulted in the identification of class 9 viruses. The movement of this virus strain, in a region previously endemic with class 2 ILTV, suggests a lapse in appropriate biosecurity measures associated with transport between farms (staff movements, feed trucks etc.).

One case in May 2009 revealed a mixed infection of class 8 and 9 ILTV strains from a farm in the Sydney basin region. There were no submissions from NSW again until April 2010, when classes 8 and 9 were found individually.

Class 2 was responsible for eight cases of ILT in both Queensland and Victoria. This class was the most prevalent in VIC during the outbreak period 2007/2008 and our laboratory records indicate that it had been in circulation in VIC as early as 1999 and QLD as early as 2001.

The presence of a class 3 ILTV isolate in game bird flocks in NSW, QLD and WA suggests a movement of the class 3 virus along with game-bird transport. Records from our laboratory indicate that this genotype was in circulation in VIC and South Australia in 2004 (10). This emphasises that ILTV can also be a problem for "back-yard" and other small-scale poultry operations, which are not usually vaccinated and may act as a reservoir for the virus in the Australian poultry population, and vice-versa.

Table 1. Classification of ILTV strains based on RFLP digestion pattern combinations

PCR products				
TK	ICP4	ICP18.5		
Restriction Enzymes				
*Msp*I	*Hae*III	*Hae*III		
RFLP PATTERNS			**CLASS**	**Vaccine strain equivalent**
A	A	A	1	A20 / SA2 (Fort Dodge)
B	B	B	2	-
B	A	C	3	-
B	C	C	4	-
A	A	A	5*	-
B	B	C	6	-
B	D	C	7	Serva (Intervet Nobilis)
A	D	C	8	-
A	D	A	9	-

* PCR-RFLP of PCR product ORFB-TK using *Fok*I, produced pattern B, which represents class 5.

This is different for that produced by SA2 being pattern A, representing class 1

Table 2. Field isolates typed in the current study over a twelve-month period.

Month/Year	Region	Flock type	ILTV Class	Number of cases
May/2009	Sydney basin, NSW	Broilers	8+9	1
August/2009	Central NSW	Game	3	1
August/2009	Gold Coast Hinterland, QLD	Broilers	2	1
September/2009	Gold Coast Hinterland, QLD	Broilers	2	2
September/2009	South Eastern QLD	Game	3	1
October/2009	Gold Coast Hinterland, QLD	Broilers	2	1
November/2009	Geelong outskirts, VIC	Layers	9	1
November/2009	Geelong outskirts, VIC	Broilers	9	2
November/2009	Geelong outskirts, VIC	Layers	9	1
December/2009	Geelong outskirts, VIC	Layers	9	1
December/2009	Geelong outskirts, VIC	Broilers	9	3
January/2010	Outer-West Melbourne VIC	Broilers	9	1
January/2010	Central VIC	Broilers	9	1
January/2010	Gippsland, VIC	Layers	2	1
February/2010	Geelong outskirts, VIC	Broilers	9	2
February/2010	Gippsland, VIC	Layers	2	1
February/2010	Central VIC	Layers	9	1
February/2010	Gippsland, VIC	Broilers	2	2
March/2010	Gippsland, VIC	Broilers	9	7
March/2010	Perth, WA	Game	3	1
April/2010	Sydney basin, NSW	Broilers	9	3
April/2010	Gippsland, VIC	Broilers	9	7
April/2010	Geelong outskirts, VIC	Broilers	9	1
April/2010	Gippsland, VIC	Layers	9	1
April/2010	Sydney basin, NSW	Broilers	8	1
			Total	45

In this study, genotyping of ILTV isolates by PCR-RFLP provided an understanding of the behaviour and spread of ILTV over the past year. The technique was adopted from a previous study (10) that screened a number of ILTV genes/genomic regions and restriction enzymes to select the most useful combination for ILTV typing. It needs to be highlighted that only a small number of genes are screened in this technique. In addition, RFLP can detect nucleotide sequence variations occurring only in the restriction sites used – there may be other variations in the genes/genomic regions that go undetected by this technique. Complete nucleotide sequences of the genes used in this study, or of other ILTV genes such as gM/UL9 and gG/UL47 12 may be useful to confirm the relatedness of the classes designated in this

study. Alternatively, complete genomic sequence analyses of ILTV isolates characterised in this study may reveal a full understanding of the relationship of the viral isolates. Information derived from full genome sequencing will also be useful for development of more rapid classification techniques such as real time PCR with high resolution melt curve analysis (HRM) or single strand conformation polymorphism (SSC) analyses.

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Of Rabbits, Chickens and Champagne – Everything Old is New Again

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The New

To investigate host related differences in virulence, two strains of *Pasteurella multocida* capsular type F were compared. The strains had been isolated from chicken and rabbit, respectively, and both caused severe disease in the relevant original host. Multilocus sequence typing demonstrated that the two isolates were clonal – both belonging to ST9. Infection experiments in both homologous and heterologous hosts showed that the chicken strain affected rabbits severely and all animals died whereas in chicken less than half of the birds showed pathological abnormalities. The rabbit strain only caused mild symptoms in chicken although it was found highly virulent in rabbit. This is the first time host specific virulence reactions have been determined by capsular type F strains of *P. multocida*.

The Old

The demonstration that chicken isolates of the organism we now know as *Pasteurella multocida* can be highly virulent for rabbits was originally made by Louis Pasteur in the 1880s. In work performed in the walled gardens of the Pomery Champagne Estate (a concept we now called bio-secure containment), Pasteur and his nephew Lior showed that rabbits exposed by several routes (including on feed) to chicken isolates of *P. multocida* suffered high mortality. These experiments were performed as preliminary work intended to help Pasteur win the incredibly valuable prize of $10,000,000 then on offer from the colonial governments of the eastern seaboard of Australia and New Zealand. Pasteur then sent his nephew to Australia to claim the prize. In a series of incidents that involved the Father of the Federation, the most famous actress of the time, rabid "anti-vaccination" forces, vested interests (who preferred their solutions of warren ripping and fencing), Pasteur was denied his claim. However, the Pasteur influence did leave a marked presence in Australia – the recognition of the presence of anthrax, the effective Pasteur anthrax vaccine, the new Australian produced anthrax vaccine (from John McGarvie-Smith who left the Pasteur team to set up his own vaccine company). The final connection was that the Queensland colonial government attempted to employ Lior as the founding director of the Institute now known as the Animal Research Institute. Lior was too valuable for Pasteur and was ordered back to France. The Queensland government employed a scientist who had spent some time in the Pasteur laboratory in Paris – C.J. Pound. Amongst the earliest work of C.J. Pound was the first confirmation that fowl cholera was present in chickens in Australia. One of arguments against the use of P. multocida as a biological control agent was that the agent was not present in Australian chickens. Pound was simply the first person to look for the organism. The confirmation of the presence of fowl cholera caused considerable controversy and several law suits that stained the otherwise glittering career of C.J. Pound.

Everything Old is New Again

Our work using MLST and PCR technologies has added some additional fine details (we have

confirmed molecular evidence of the clonality of our isolates and that the isolates both belong to capsule type F). In addition, we examined our isolates in both the original host and the alternative host. However, our findings that the chicken isolate can indeed cause disease in rabbits is simply confirmation of knowledge acquired more than 100 years ago at the very dawn of the science of microbiology by Pasteur and his colleagues.

Recommended Reading

Stephen Dando-Collins (2008) Pasteur's Gambit – Loius Pasteur, the Australasian rabbit plague and a ten million dollar prize. Vintage Books, Australia.

Salmonella and Campylobacter Control in Processing

Dr Margaret A. MacKenzie

Inghams Enterprise Pty Limited

Campylobacter and Salmonella are the two most frequently reported foodborne diseases, and chicken meat is an important food vehicle.

Public health agencies worldwide are intensifying their efforts to lower foodborne illness by developing standards and control strategies aimed at eliminating or reducing these pathogens in the food chain, and setting goals for human salmonellosis and campylobacteriosis.

On farm control strategies based on biosecurity will continue to reduce Salmonella and Campylobacter incidence, however at present complete eradication of Salmonella and Campylobacter is unrealistic.

Risk assessments indicate that high levels of pathogens on chicken meat constitute the major public health risks. A reduction in the levels of Salmonella and Campylobacter on chicken would be expected to lead to a reduction in the number of human cases.

The number and prevalence of Salmonella and Campylobacter on poultry entering the processing plant varies greatly depending on the time of year, region, flock, environment and preharvest management. Recent Australian research showed Salmonella levels ranging from log 2.8 – 4.9 and Campylobacter from log 8 – 8.5 on incoming carcases.

The levels on carcases leaving the plant can increase or decrease depending on the effectiveness of process control strategies in the processing plant.

Poor process control will increase both the prevalence and numbers of Salmonella and Campylobacter on carcases due to multiplication and cross cross-contamination.

The implementation and adherence to pathogen reduction interventions based on HACCP principles during processing can effectively lower the levels of Salmonella and Campylobacter to target levels of $\leq$ log 2.0 for Salmonella and $\leq$ log 3.78 for Campylobacter on carcases exiting the chiller. At these levels risk assessments indicate there would be a significant reduction in human illness from chicken consumption.

In the 2009 Australian industry baseline survey of carcases ex chill, prevalence of Salmonella was 18% (8% non-sofia isolates), overall average count was 2.09 log MPN per carcase and range 1.18 – 2.88 log MPN per carcase, prevalence of Campylobacter was 95%, overall average count was log 3.93 per carcase, and range 2.52 – 7.4 log per carcase. Overall, 89% of plants achieved the Salmonella target of $\leq$ log 2.0 and 67% achieved the Campylobacter target of $\leq$ log 3.78.

Critical control points in the processing plant for effective pathogen reduction include plant hygiene and sanitation feed withdrawal time (8 to 12 hours), prescald brushes and sprays, scald tank hygiene (multiple counterflow tanks, adequate overflow rates, scald treatments), evisceration operation and adjustment to prevent faecal leakage, multiple washes and sprays, immersion chill disinfection (5ppm free available chlorine, pH 6.0 – 6.5, adequate overflow rate, temperature $\leq$ 4°C, counterflow current, meat : water ratio, prechill immersion or spray disinfection, airchill sprays, postchill dips and sprays and postchill hygiene and temperature control ($\leq$ 4°C).

Monitoring of Salmonella and Campylobacter on carcases ex chill should be used to verify the effectiveness of process control during processing and compliance with industry or regulatory targets.

Processing plant pathogen intervention strategies, operated correctly, can achieve a significant reduction in Salmonella and Campylobacter levels on broiler carcases which is likely to reduce the incidence of Salmonellosis and Campylobacteriosis foodborne illness in humans.

SNP Types of *Campylobacter jejuni* Isolated from Different Hosts

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Summary

In this study we applied Single Nucleotide Polymorphism (SNP) typing to 577 *Campylobacter jejuni* isolates from different host species, including chickens, feedlot cattle, dairy cattle, dogs, cats and humans. We found that SNP typing was an effective method for genotyping *C. jejuni*. The results show that some *Campylobacter* genotypes do show host specificity, which should be considered when investigating outbreaks of campylobacteriosis in humans. The SNP typing results clearly show that there are sources other than poultry meat associated with human campylobacteriosis.

Introduction

Campylobacter is the major cause of human gastrointestinal illness in Australia, with rates of infection approximately double those for *Salmonella* spp. each year. While poultry are a significant source of these infections, there is a considerable body of evidence that there are other sources, for example raw milk and pets (1, 2). Some types of *C. jejuni*/*coli* appear host specific (meaning that some types occur only in chickens) while other types can be found in multiple hosts (e.g. in both chickens and humans). There is a general agreement that the definitive method for typing *C. jejuni*/*coli* is Multilocus Sequence Typing (MLST) (3-5). The high cost of MLST, caused by the requirement to sequence around 500 base pairs in each of seven genes, has recently been overcome by the use of a combination of kinetic PCR and interrogative data analysis that provides the power of conventional MLST but at a much lower cost and with a more rapid response time. This new technology is called Single Nucleotide Polymorphism (SNP) analysis.

The objective of this study was to apply the technique of SNP analysis for typing *C. jejuni* to both poultry and non-poultry isolates. To achieve this we extended the current Agri-Science Queensland *Campylobacter* collection to include poultry isolates from other research groups and industry and we obtained non-poultry isolates from sources such as cattle, pets and humans. We were then able to compare and contrast the SNP types of the poultry *C. jejuni* with the non-poultry types.

Materials and Methods

In our study SNP typing as previously outlined (6) was applied to 577 *C. jejuni* isolates. The isolates consisted of:

- 32 Chicken faecal/caecal isolates from epidemiological studies (1999-2003)
- 36 Chicken Factory isolates (2008)
- 76 Chicken Factory isolates (2005-2006)
- 77 Chicken caecal isolates collected from a national survey (2003-2004)
- 93 Dairy Cattle isolates (2006-2008)
- 123 Feedlot Cattle isolates (2006-2008)
- 39 Dog and eight Cat isolates (2006-2008)
- 46 Human isolates (2000)
- 47 Human isolates (2008).

Results

In our study we applied SNP typing to 577 *C. jejuni* isolates from different host species. The 577 isolates were grouped into 39 different SNP types. Our results show that some genotypes are associated with multiple host species whereas other genotypes are predominantly associated with limited host

species. As an example, SNP type 44 was a genotype found only in humans, dogs and cats. SNP typing has also shown subtle differences in genotype distribution. SNP type 5 was associated with dairy cattle while SNP type 13 was associated with feedlot cattle.

Discussion

SNP typing is an effective method for genotyping *C. jejuni*, including the capacity to recognise host associations. Some *Campylobacter* genotypes do show host specificity, which should be considered when investigating outbreaks of campylobacteriosis in humans. The genotyping results clearly show that there are sources other than poultry meat associated with human campylobacteriosis. In particular, pets need to be considered as a source of *C. jejuni* for humans. Some preliminary data from our studies indicates that regional and company influences may play a role in the genotype distribution of *Campylobacter* isolates in poultry and we are currently investigating this further.

Overall, SNP typing has been shown to be a convenient first line tool for screening *C. jejuni* isolates. It is user friendly, easily transportable between research groups, is relatively cheap and has the advantage that it is directly linked to MLST. Unlike PFGE, SNP typing lends itself to robotics for sample preparation and assay set up. We would recommend SNP typing as a front line typing method when investigating outbreaks of campylobacteriosis or when looking for host associations with particular genotypes of *C. jejuni.*

Acknowledgments

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Evaluation of Residues in Eggs after Treatment of Pullets with Toltrazuril, Sulfadimidine or Amoxycillin.

Timothy Wilson[1], Angus Crossan[2] and Peter Scott[1]

Background

There is a limited number of therapeutics available in Australia for treating disease in layer pullets. In fact without the provision of specific egg residue data to the regulator any product registered for chickens must have the disclaimer; "Not to be used in poultry producing eggs for human consumption or which will ever produce eggs for human consumption". The absence of adequate antibiotic classes has welfare implications in limiting the ability to teat clinically sick birds under particular circumstances as well as impact lifetime productivity.

In 2004 the APVMA (Australian Pesticides and Veterinary Medicines Authority) granted AECL (Australian Egg Corp Ltd) permits for the use of 3 groups of antibiotics in replacement pullets (both layers and breeders) pending the outcome of egg residue data to allow the establishment of suitable withholding periods to allow further use in a manner shown to be safe to the public. Scolexia Avian and Animal Health Consultancy was contracted by AECL to undertake the study reported here.

Experimental design

The hypothesis examined the MRLs and withholding periods allowed under the APVMA permits as follows:

For each of the therapeutics ("y"):

1. H0 Egg [Antibiotic residue] ≤ MRL y at the end of the withholding period y

2. H1 Egg [Antibiotic residue] > MRL y at the end of the withholding period y

The temporary MRLs and withholding periods in eggs for each antibiotic are listed below;

- toltrazuril 0.05 mg/kg 6 weeks
- sulfadimidine 0.01 mg/kg 2 weeks
- amoxycillin 0.01 mg/kg 1 week

In addition the rate of decline of residues in all eggs was logged and the concentration of the residue in first eggs was described with respect to time since treatment.

Materials and Methods

Treatment groups were selected after discussion with the APVMA. For the amoxycillin and toltrazuril groups one commercial product was selected as representative. For the sulphonamide group, sulfadimidine was selected as representative of the group for two reasons; it has the lowest MRL of the group and is contained in products with the highest dose rates. Treatments were administered as described in table 1 below.

As toltrazuril may be used on more than one occasion, treatments (each for 2 days) were given one week apart. Treatments for amoxycillin and sulfadimidine were each given daily for 5 days. Hisex pullets were transferred to the cage layer trial facility (Scolexia Animal Research Facility (SCARF) at 12 weeks of age. They were fed a commercial bagged ration that contained no antibiotics.

Table 1: Treatment groups

Group	Treatment	Birds per group	Dose rate (active) mg / kg BWt / day
A	Control	33	
B	Baycox® Coccidiocide Solution (Toltrazuril)	30	7
C	CCD Sulfadimidine sodium soluble Sulfadimidine sodium	30	45.6
D	AFS Amoxycillin soluble Amoxycillin trihydrate powder Amoxycillin trihydrate	30	20

The treatments were administered such that the withholding periods (listed above) for each group ended on the same day. The point of lay was managed by a lighting program which slightly delayed the onset of lay to ensure most "first eggs" were laid around the end of the withholding period. Each treatment was mixed in water and administered orally. Prior to treatment birds were weighed. For sulfadimidine and amoxycillin treatments the birds were re-weighed on the forth day of treatment. The birds were weighted on the first day of each toltrazuril treatment. Doses for each bird were calculated according to bodyweight for that bird

Table 2 lists the residue definitions and anolytes that were examined. The analysis was conducted using HPLC/MS/MS by Agrisearch Analytical (Victoria Rd, Roselle, NSW). Egg shell was not included in the analysis which analysed yolk and white combined.

Table 2: APVMA MRL Standard Anolytes to be determined.

Antibiotic	Residue definition	LOQ (mg/kg)
Toltrazuril	Sum of toltrazuril, its sulfoxide and sulfone, expressed as toltrazuril.	0.05
Amoxycillin	Inhibited substance, identified as amoxycillin	0.01
Sulfadimidine	The parent compound *per se*.	0.01

Results

Egg-laying began in one bird on the 14th of November 2010 and by the 17th of November at least one bird from each group had begun to lay. Over a third of the treated birds (33/90) laid their first egg three days either side of the end of the withholding period. Results of "first eggs" as well as serial results (approximately every two days) from 5 chickens are presented for each group.

In all group B assays toltrazuril and toltrazuril sulfoxide residues (if present) were below the limit of detection. Quantifiable levels of toltrazuril sulfone were found in some of the treated eggs. Table 3 lists the results.

Days after last treatment	Bird No	Total Toltrazuril (mg/kg)	Days after last treatment	Bird No	Total Toltrazuril (mg/kg)
31	29	<0.073	44	10	<0.02
34	27	<0.074	45	6	<LOD
36	1	<0.068	46	2	<0.019
36	19	<0.043	46	3	<0.016
37	8	<0.033	46	18	<LOD
37	12	<LOD[1]	47	15	<LOD
37	13	<0.019	47	17	<0.021
37	23	<0.017	48	5	<0.015
40	14	<LOD	48	11	<0.021
42	7	<0.028	48	21	<LOD
42	20	<0.016	49	4	<LOD
42	24	<0.02	53	16	<LOD
42	26	<0.019	55	22	<LOD
43	28	<0.019	56	25	<0.017

Table 3: Concentration of total Toltrazuril in first eggs since treatment

1 LOD = Limit of detection

The temporary MRL for total toltrazuril in eggs examined under the hypothesis was 0.05 mg/kg with a 6 week egg withholding period. The last day that a first egg was over the MRL was day 36 after treatment. By day 38 the average residue in eggs tested serially from the same five chickens were under the MRL. The concentration of toltrazuril residues in first eggs on the forty third day after treatment or thereafter was never greater than 0.021 mg/kg and on the 42nd day after treatment no egg had a value of higher than 0.036 mg/kg. The average of all eggs tested 42 days after treatment was 0.018 mg/kg total toltrazuril. Therefore the null hypothesis that the concentration of toltrazuril in eggs after a withholding period of 42 days is under the MRL was supported.

This study demonstrates that multiple uses of the anticoccidial product toltrazuril in chicken pullets will not result in residues in eggs over the MRL of 0.05 mg / kg total toltrazuril if a withholding period of 42 days (6 weeks) after treatment is observed.

Sulfadimidine residues in the first eggs from pullets in group C are listed below in table 4

Bird no	DALT[1]	Sulfadimidine (mg/kg)	Bird no	DALT[1]	Sulfadimidine (mg/kg)
51	4	0.36	37	13	<LOD
48	7	0.1	54	13	<LOD
52	7	0.081	56	13	<LOD
58	7	0.011	34	14	<LOD
31	9	<LOD	41	14	<LOD
39	9	<LOD	57	14	<LOD
45	9	<LOD	47	15	<LOD
33	10	<LOD	55	15	<LOD
42	10	<LOD	35	16	<LOD
44	10	<LOD	46	16	<LOD
33	11	<LOD	38	19	<LOD
40	11	<LOD	50	19	<LOD

60	12	<LOD

Table 4: Sulfadimidine residue in first eggs from pullets treated with sulfadimidine sodium
Nb: LOD = Limit of detection

1 DALT = days after last treatment

The temporary MRL in eggs examined under the hypothesis was 0.01 mg/kg, with a temporary withholding period of 2 weeks. All first eggs tested were under the temporary MRL by 9 days after the last treatment and all eggs collected from 5 hens serially were under the temporary MRL by 10 days after treatment. In both groups the sulfadimidine residues in eggs were under the limit of detection by day 10. The null hypothesis that the sulfadimidine residues in eggs from pullets treated with sulfadimidine would be under the temporary MRL by the end of the withholding period of 14 days is supported by the data.

Quantifiable residues of amoxycillin were found in some of the eggs from group D birds. Amoxycillin residues in first eggs are listed below in table 5.

Table 5: Amoxycillin levels in first eggs from pullets treated with Amoxycillin

Bird number	Days after last treatment	Amoxycillin (mg/kg)	Bird number	Days after last treatment	Amoxycillin (mg/kg)
67	-2	0.013	75	5	<LOD
73	-1	0.009	86	5	<LOD
64	0	0.028	61	6	<LOD
81	1	0.016	71	6	<LOD
83	1	0.006	79	6	<LOD
90	1	0.02	66	7	<LOD
62	2	0.008	70	7	<LOD
63	2	0.013	84	7	<LOD
77	4	<LOD	65	8	<LOD
80	4	<LOD	68	8	<LOD
82	4	<LOD	74	8	<LOD
85	4	<LOD			

Nb: some values listed were below the limit of quantification LOD = Limit of detection

The temporary MRL examined under the hypothesis was 0.01mg/kg with a temporary withholding period of 7 days. No first eggs were collected on day 3 after the end of treatment and on day 4 and thereafter all first eggs had no detectable amoxycillin residues. On day three after the end of treatment average amoxycillin residues in eggs from five birds were on average 0.0033 mg/kg and from day four and onwards no amoxycillin residues were detected in the eggs from any of the five pullets. The null hypothesis that residues of amoxycillin in eggs from treated hens would be under the MRL after a withholding period of 7 days is supported by the data in this study.

The results of the study for each of the three anolytes, toltrazuril, sulfadimidine and amoxycillin support the null hypotheses that when treated at the maximum dose rate eggs from birds treated with these antimicrobials will be under the MRLs examined if the prescribed withholding periods for eggs are observed.

The Poultry CRC and the RIRDC – Current Research Topics and Their Objectives

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Introduction

This paper seeks to set out the broad focus of the two organisations associated with the funding of poultry research in Australia. The focus of the paper is to set out the general features of the research programs of the two bodies – the Poultry CRC and the Chicken Meat program within the Rural Industries Research and Development Corporation (RIRDC)

The CRC

The new Poultry CRC officially came into life on January 1st 2010. The CRC has three integrated programs of research. Program 1 is entitled Health and Welfare; Program 2 is entitled Nutrition and Environment while Program 3 is entitled Safe and Quality Food Production. At the time of writing, the formal decisions on which particular sub-projects (the CRC equivalent of an RIRDC Project) will be funded have not been made. However, the formal documentation for the CRC does provide broad outlines of the outputs expected from the three Programs. These outputs include:-

Program 1

- Novel vaccines for significant diseases
- Novel diagnostic tests
- Methods for sex determination in poultry
- Bacterial and viral vectors tested for delivery of NetB vaccine
- Novel anti-viral and anti-parasitic therapeutics
- New evidence-based welfare methods

Program 2

- A set of nutritional tools to maintain good gut health in poultry
- New feed formulations based on net energy of common Australian feed ingredients
- Commercialisation of odour and greenhouse gas (GHG) sensing systems and mitigation processes

Program 3

- Rapid detection methods for *Campylobacter* in poultry
- Management options that allow industry to adopt targeted, operation specific food safety programs to reduce Campylobacter levels in meat chickens
- Improvements in egg quality and safety

While no longer a separate program, education is a strong theme within the CRC. There are plans for an active post-graduate program. As well, direct industry associated education activities (VET, industry placements, internships) will have a high priority.

The RIRDC

The RIRDC has, at the time of writing, a total of 38 projects that are listed as current. The projects are at varying stages of the project cycle – from just commencing to awaiting approval of the final report. An overview of the broad areas of activities of these projects is in Table 1:-

Broad Area	Project Topics
Conference Sponsorship Conference Travel (Four projects)	• AI • *Campylobacter*, • PIX
Infectious Diseases (Eight Projects)	• *Chlamydophila* • *Pasteurella multocida* • AI • IB • IBD • ILT • MD
Food Safety (Eight Projects)	• *Campylobacter* - abattoir surveys/workshops, effect of litter re-use, phages to control levels in live birds, rapid detection, typing, vaccines • *Salmonella* - abattoir surveys/workshops, effect of litter re-use, typing
Environment (Eight Projects)	• Litter – broad-acre usage; biochar workshop; nutrient run-off, alternative fertiliser • Lifecycle Assessment • Mass Mortality Composting • Waste into Energy • Artificial Olfaction
Nutrition (Four projects)	• Heat stress • Assessing available energy in grains • Steam pelleting of sorghum • New triticale lines
Others (Six projects)	• Workboot Series • Biosecurity/Food Safety DVDs • Darkling Beetles • Nutrient composition of chickens • Human resources audits • Program Evaluation

Table 1: Overview of the broad areas of activities of the RIRDC

Roles of RIRDC and CRC

The Australian poultry industry is in a unique position in the world. The industry has available two significant mechanisms of industry focussed, government supported research programs. These two programs have significant overlap in terms of research service providers as well as those making the decisions about which particular topics should be funded. This results in a co-ordinated research programs with each funding arm being aware of the activities and focus of the other body. A good example of the co-ordination is in the food safety area where the CRC and the RIRDC have agreed that the CRC program should focus attention to on-farm interventions as the RIRDC structure is more suited for in-plant interventions.

The CRC has a mandate to work as a "family" organisation. All sub-projects are led from within the "family" and all work – where possible – is undertaken within the family. The CRC has a mandate to undertake a percentage of blue sky research (with the recognition of the NetB toxin of *Clostridium perfringens* being an outstanding example of blue sky research that has significant practical applications. A similar breakthrough example is the work of the CRC on sex determination – a project which has major economic and welfare implications. The blue sky research of the CRC is balanced by

practical industry focussed work – with the suite of rapid molecular tests available from the University of Melbourne being a good example of this.

The RIRDC has a broader mandate and provides the industry with access to all interested, capable and relevant research organisations. The strong industry linkages of the RIRDC are well suited to those research programs where practical, applied research with a short horizon is required. The RIRDC has been very active recently in education and industry focussed workshops. The Workboot Book on the meat chicken industry (also associated with the CRC) has proven a very effective communication tool (it is shortly expected to reach the New York Times best seller list!!). A particularly successful recent activity has been a series of workshops focussed on interventions in the processing plants to reduce levels of *Salmonella* and *Campylobacter*.

Overall, the two research mechanisms that support the chicken meat industry are quite distinct but – at the same time – quite co-ordinated. The bodies differ in their focus and their mandates. The Australian poultry industry will gain substantial benefits from both organisations over the next five years.

Diagnostic Technologies Available to the Australian Poultry Industry

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The outcomes of an Australian Poultry CRC project have facilitated the establishment of a core diagnostic centre for poultry diseases in Australia. This laboratory can provide rapid and reliable world-class diagnostic services for the Australian poultry industry. Examples of the diagnostic assays are overnight diagnosis and strain identification for infectious bronchitis virus, *Chlamydophila psittaci*, *M. gallisepticum*, fowl adenoviruses, etc. This will enable the core centre to rapidly solve, at a low cost, epidemiological questions related to major poultry diseases in Australia. Twenty-seven diagnostic assays for different pathogens are available to the Australian Poultry Industry as follows overleaf:

:

DIAGNOSTIC ASSAYS

Test	Result
Avian Encephalomyelitis Virus (AEV) rt-PCR	detection
Avian Leukosis Virus (ALV) rt-PCR	detection
Avian Leukosis Virus Subgroup-J (ALV-J) rt-PCR	detection
Avian Nephritis Virus (ANV) PCR	detection
Avibacterium paragallinarum PCR	detection
Chicken Anaemia Virus (CAV) PCR	detection
Chlamydia sp. PCR	detection
Chlamydia sp. PCR-HRM	detection and species ID
Escherichia coli multiplex PCR	detection and virulent strain ID
Egg Drop Syndrome (EDS) PCR	detection
Fowl Adenovirus (Inclusion Body Hepatitis) PCR HRM curve analysis	detection and strain ID
Fowl/pigeon POX PCR	detection
Infectious Bursal Disease Virus (IBDV) rt-PCR-HRM	detection and strain ID
Infectious Bronchitis Virus rt-PCR-HRM	detection and strain ID
Infectious Bronchitis Virus Subgroup-1 PCR	detection and strain ID
Infectious Laryngotracheitis virus PCR	detection
Infectious Laryngotracheitis virus -PCR-RFLP	detection and strain ID
Marek's Disease Virus PCR	detection
Mycoplasma gallisepticum PCR	detection
Mycoplasma gallisepticum PCR-HRM	detection and strain ID
Mycoplasma anatis PCR	detection
Mycoplasma meleagridis PCR	detection
Mycoplasma synoviae PCR	detection
Mycoplasma synoviae PCR-HRM	detection and strain ID
Pasteurella multocida PCR	detection
Avian Reovirus rt-PCR	detection
Reticuloendotheliosis virus PCR	detection

rt: reverse transcriptase, HRM: high-resolution melt, RFLP: restriction fragment length polymorphism

Current status of Inclusion Body Hepatitis and Fowl Adenoviruses in Australian Poultry

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INTRODUCTION

Inclusion body hepatitis (IBH) in chickens emerged in 1963 in the U.S.A. (1) and was first reported in Australia in 1974 (2). The disease now has worldwide distribution in domestic avian species of all ages, with indications that incidents of the disease are increasing (3). IBH can be characterised by sudden onset of mortality, usually between 3-7 weeks of age and enlarged mottled and friable livers (4). In Australia IBH outbreaks have occurred in chickens less than 3 weeks of age (5). The economic significance of FAdVs is unclear due to the variability in their disease association (3), however with peak mortalities as high as 30% (4), adequate control measures to reduce the incidence of IBH are imperative.

FAdVs are readily transmitted horizontally via the oral-faecal route, due to high viral titres present in the faeces. Vertical transmission of FAdVs and the establishment of latent infection has been shown to occur in chickens (6), and may remain undetected for at least one generation in a specific-pathogen-free (SPF) flock (7). Immunosuppression resulting from infection with infectious bursal disease virus (IBDV) or chicken anaemia virus (CAV) have long been suspected of facilitating outbreaks of IBH (8, 9). There is however, mounting evidence that IBH can be a primary disease of poultry (4, 5, 10-12).

Previous studies of IBH outbreaks in Australia identified serotypes in species groups A (FAdV-1) and E (FAdV-6, 7 and 8) (5), with FAdV-8 exhibiting the highest virulence. IBH was a significant problem in broiler chickens from the 1970's through early 1990's, with FAdV serotype 8 (now divided into 8a and 8b) isolated from the majority of outbreaks (12, 13). Serotype 2/12 (now serotypes 2 and 11) was reported in a 12 week old SPF cockerel (14).

The Australian FAdV vaccine (Intervet, Pty Ltd.) was developed in 1989 from a non-attenuated FAdV-8b strain (Esurient) and aimed to control IBH by means of protecting broiler flocks via maternal antibodies from vaccinated parent flocks. The vaccine is known to prevent IBH in progeny birds aged mainly 7-21 days, but it is unlikely that it prevents IBH after three weeks of age (personal communications with Tom Grimes). In recent years there have been sporadic occurrences of IBH in Australian broilers. Investigation of the re-emerging problem of IBH has been undertaken to identify the FAdV serotype(s) involved, and to establish if FAdV was the sole causative agent in the disease in the absence of immunosuppression caused by IBDV or CAV.

RESULTS AND DISCUSSION

Identification of the serotype(s) involved in IBH outbreaks is very useful for epidemiological tracing and is of critical importance where vaccination is to be used for control of disease (3). Typing of strains conventionally by virus microneutralisation is cumbersome, requiring reference materials, and results often require extensive interpretation (11). Tests using PCR together with DNA sequencing (15) and/or restriction enzyme

analysis (16, 17) have been used for comparatively faster FAdV typing, but the process is lengthy and results can be difficult to interpret. Recently, HRM curve analysis of PCR amplicons of the Loop 1 (L1) region of the hexon gene was used to accurately genotype reference strains from each of the twelve FAdV serotypes (18), and has since been introduced as a commercially available test at the APCAH diagnostic laboratory at The University of Melbourne for routine typing of field specimens.

Serological screening of parent flocks and progenies for type-specific antibodies may elucidate the presence and distribution of different FAdVs, and the efficacy of current vaccination strategies. Serum microneutralisaiton is an option, however this is costly and time consuming to perform as a routine diagnostic test. Attempts to express a protein suitable for use in a type-specific ELISA for rapid and affordable serological screening have so far been unsuccessful. This may have been due to incompatibility of the expression system used with the FAdV protein and/or the presence of hydrophobic regions in the protein. Codon optimisation or a different expression system may be required for successful expression of the FAdV hexon proteins, for the development of a type-specific ELISA.

Since 2000, 42 cases from commercial poultry farms located in Victoria, New South Wales and Queensland have been submitted to the APCAH laboratory. Nine of these cases were confirmed as IBH by histopathology alone. Thirty-three cases were screened by PCR/HRM genotyping, with FAdV-8b identified in 13 cases and FAdV-11 identified in 19 cases. In one case FAdV-1 was also identified, in the absence of clinical signs or histopathological lesions relevant to IBH in this individual bird. In the five cases tested for CAV and IBDV, neither virus was detected in association with an IBH outbreak. Evidence suggested sufficient antibodies against CAV and IBD were present in parent flocks and there was no indication that immunosuppression caused by these viruses was present in any of these cases.

The Australian FAdV vaccine is registered to be administered at 18-20 weeks of age by eyedropper to 100% of the flock at the rate of one label dose per bird. However, the vaccination strategy used by the five poultry companies for broiler parent flocks varied. All parent flocks were vaccinated once, twice or three times, usually via the drinking water, at between 9 and 18 weeks of age, with the flock receiving 10, 20, 50 or 100% of the recommended dose on each occasion. Where 100% vaccination was used, only FAdV-11 was detected in IBH cases in progenies. No relationship could be drawn between geographical location and the FAdV serotype identified from outbreaks of IBH, nor was there any correlation between FAdV serotype and rate of mortality.

Chickens from a wide range of ages, 9 to 37 days, were affected. All bar one of the cases from which FAdV-8b was isolated were from broilers over 17 days of age. It may be postulated that these were the result of horizontal infection, and a lack of sufficient protective maternal antibodies against FAdV-8b. However, FAdV vaccination of parent flocks in the absence of uniform or high antibody may result in vertical transmission of the vaccine virus to the progeny.

In all of the cases affecting birds under 16 days of age, except one, the FAdV-11 field strain was isolated. These cases may be a result of vertical transmission of the virus from the parent flock. However, it is also possible that they resulted from horizontal infection, particularly since cross-protection may not exist between the Australian vaccine and this field strain, as these viruses belong to different species groups. While cross-protection may exist between serotypes belonging to the same species group, this is unlikely to be the case for serotypes of different species (3).

These investigations of recent outbreaks of IBH in Australian broiler flocks provide evidence that it is a primary disease resulting from two alternative FAdV strains from different species groups. The route of infection, horizontal or vertical, is unclear. Consequently, protection against outbreaks of IBH in Australian broiler flocks may require the use of an additional FAdV-11 vaccine, or a single dual serotype FAdV vaccine, and should be further investigated. The application of a killed vaccine, which is common practice in other countries where IBH is prevalent, or attenuation of live viruses for use in vaccines, should also be considered.

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An Examination of the Effect of the Homeopathic Preparations, Fowlpox Isode, and *Thuja Occidentalis*, on Production and Immune Parameters of Lohmann Brown Laying Hens Before and After Fowlpox Vaccination.

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Summary

Fowlpox virus has economic importance, particularly in developing countries. Research was undertaken to see if homeopathic preparations would have significant effects on bird immune and production response to Fowlpox vaccination, and if the use of homeopathic medicines would be worth further research. Responses to medication with homeopathic Fowlpox Isode and homeopathic *Thuja occidentalis* were examined in healthy Lohmann brown laying hens before and after Fowlpox vaccination. After layer vaccination hens showed significant ($P<0.05$) body weight change in the Fowlpox Isode treated group compared to controls, and increased vaccine site swelling in the *Thuja occidentalis* treated group. Therefore homeopathic preparations altered vaccine response in healthy Lohmann Brown hens, and further research is justified.

Key words: Fowlpox vaccination, Fowlpox Isode, Immune, *Thuja occidentalis*, Homeopathic Lohmann Brown, Production.

Abbreviations: FWPV: Fowlpox virus, FPI: Homeopathic preparation Fowlpox Isode, HP: Homeopathic preparations, LB: Lohmann brown hen, Thuja: Homeopathic preparation *Thuja occidentalis*, TW: Tap Water, Water BP: Water and ethanol British pharmacopeia, Im: Imitation treatment.

Introduction

Fowlpox (FWPV) is an Avipox virus (1) and is the only acute Pox virus which affects laying hens. In developing countries FWPV may have economic importance second only to Newcastle disease as, for in many developing countries, flock vaccination and biosecurity are expensive.

Homeopathic preparations (HP), which are inexpensive and easy to administer, have been used in the amelioration of the effects of Pox viruses in humans and animals (2, 3), and in the modification of response to viral disease in animals (4). The possibility of using HP to improve animal production and FWPV disease control and treatment led to research in the Lohmann Brown (LB) which is a common backyard and free range hen in southern Queensland, as well as a high producing commercial hen in Europe. This trial was put in place to examine effects of two homeopathic preparations *Thuja occidentalis* (Thuja) and FWPV Isode (FPI) on the immune and production parameters of Lohmann Brown hen in a controlled situation before and after FWPV vaccination. The trial studied birds in individual cages.

Materials and Methods

Four treatment groups of twenty-seven individually caged Lohmann Brown hens (LB) were each given one drop of the following by beak from five to 14 weeks of age: the first treatment was the homeopathic preparation *Thuja occidentalis* (Thuja) made from the plant extract, the second was Fowlpox Isode (FPI) made from the Webster's' Fort dodge Fowlpox Vaccine, the third was made from the standard homeopathic diluent of mixed water and ethanol, British Pharmacopeia (Water BP), a fourth was Tap Water (TW). In a fifth treatment of 27 birds the handling process of dosing was simulated: imitation treatment (Im).

The FPI was a homeopathic preparation and should not be confused with the Fowlpox virus (FWPV) live vaccine from which it was made. A 30c potency was used weekly for 3 weeks before the first FWPV and a 200c daily for 3 days before the second FWPV. A c potency is a one in a hundred dilution of the medicine with Water BP. Egg numbers, body weights, feed consumption and body temperature were measured. Inoculation site thickness was measured after FWPV at 75 and 131 Days (age). Phytohemagglutinin injection response was measured two weeks after vaccination.

Results

Body weight gain of Fowlpox Isode treated birds increased ($P<0.05$) at three weeks after layer vaccination, and comb swelling showed greater fluctuation in *Thuja occidentalis* treated birds than all others two and four days after the layer vaccination.

Table 1: Least Square Means (LSM) differences in BW between 130-146 Days (age), two weeks after Fowlpox vaccination and 130-153 Days, three weeks after Fowlpox vaccination ± SE

LSM BW Difference g	**Treatment ± SE**				
Days	TW	Water BP	FPI	Thuja	Im
130-146	-1.4 ± 5^{ac}	-14 ± 4^{b}	-11 ± 6^{ab}	2 ± 4^{c}	-9 ± 5^{ab}
130-153	-3 ± 5^{a}	-5 ± 4^{a}	12 ± 6^{b}	-12 ± 4^{a}	-9 ± 5^{a}

Values with different letters ^{a,b} are different ($P<0.05$), Means are from 810 estimates. T Water: Tap water from university supply, Thuja: HP preparation made from *Thuja occidentalis*, Water BP: HP preparation made from water and ethanol British pharmacopeia, homeopathic diluents, FPI: Homeopathic preparation made from FWPV vaccine, Im: handling alone, imitation dosing, no medication.

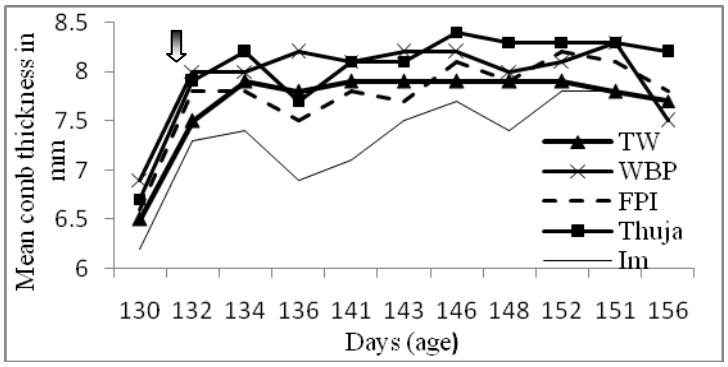

Figure 1: Least Square Means (LSM) comb thickness (mm) 131-156 Days (age),
Fowlpox vaccination 131 Days, ± SE TW 1.1, WBP 1.6, FPI 1.1, Thuja 3.7, Im 1.3
Means were based on 4,455 estimates. T Water: Tap water, Thuja: HP preparation made from *Thuja occidentalis*, Water BP: HP preparation made from water and ethanol British pharmacopeia, FPI: Homeopathic preparation made from FWPV vaccine, Im: imitation dosing, Arrow marks Fowlpox vaccination.

At 134 days there were differences due to treatment ($P<0.01$). Thuja combs were thicker than Im ($P < 0.01$), but not different to the other treatments, which were similar to one another ($P>0.05$) and Im. At 132 and 134 days soft swelling in the Thuja group made measurement difficult, denoted by high SE.

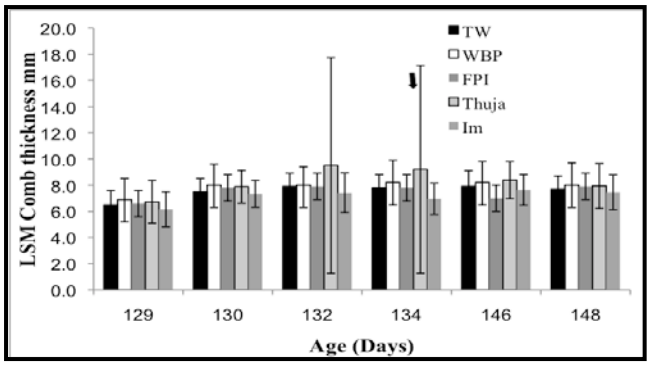

Figure 2: Least Square Means (LSM) comb thickness (mm) 129-148 Days (age) ± SE

Means were taken from 2430 estimates. T Water: Tap water, Thuja: HP preparation made from *Thuja occidentalis*, Water BP: HP preparation made from water and ethanol British pharmacopeia, FPI: Homeopathic preparation made from FWPV vaccine, Im: handling alone. Arrow shows time of significant difference.

Other parameters measured did not show significant changes although there were numerous trends which were not significant, but may be so, should there be further research with larger groups of birds.

Discussion

Results showed that HP can affect hen responses to FWPV vaccination despite large individual hen variation. This means that effects of HP are worth further research with larger numbers of birds to reduce variation.

Future trials could occur in different commercial situations. Measurement could include full blood counts, antibodies, and cytokines (interferon) (1). Blood could be sampled after ovalbumin, sheep red blood cell and bovine serum inoculation (5, 6), and before and after exposure to FWPV, Avian encephalitis or ILT, and Marek's disease. Histopathology of inoculation sites after vaccination (7) could be examined. As well, increasing the potency of homeopathic preparations to 1M or 50 M (one in a 100, a thousand times, one in a hundred, 50 thousand times) may provide greater change.

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Chicken Infectious Anaemia Virus: An Insidious Problem For Conventional and SPF Poultry Producers

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Chicken infectious anaemia virus (CIAV or CAV) has been recognized as a pathogen since 1979, but the virus has been present in the USA at last since 1959 (12). The economic impact of the virus on commercial poultry operations is often poorly recognized unless newly hatched chicks without maternal antibodies become infected before two weeks of age. In the majority of commercial flocks infection occurs after maternal antibodies wane and subclinical immunosuppression may precipitate or enhance disease by other pathogens. Protection against clinical infection is achieved by vaccination of parent flocks if these have not seroconverted between 9 to 12 weeks of age by natural infection (8,9). Vaccines are also available for broilers in the USA, but these have not been authorized for use *in ovo* or in one-day-old chicks. There are two important problems in developing live CIAV vaccines for *in ovo* or in newly hatched chicks. The first one is that maternal antibodies interfere with the replication of vaccine virus and development of active immunity, which is similar to the situation with infectious bursal disease (IBD) vaccines. To overcome this problem immune complex vaccines have been developed for the control of IBD. We have used a similar approach for CIAV. We found that certain combinations of antigen and antibodies delay virus replication and induce an immune response in the absence of anaemia when used in maternal antibody-free chicks (10). The second problem is how to test for vaccine efficacy when vaccinated birds are challenged after 2 weeks of age. Using differential quantitative PCR with primers specific for either the vaccine or challenge virus we showed vaccine-induced protection against replication of the challenge virus (10). Additional studies are needed to determine if immune complex vaccines for CIAV are protective under field conditions and economically viable.

Problems with CIAV infections in specific-pathogen-free (SPF) flocks occur frequently and often without apparent reason. Most often seroconversion is noted in a few birds shortly before or after the onset of egg production. During the last 12 years we have studied the problems of CIAV infections in closed SPF flocks maintained by Cornell University in a filtered-air, positive-pressure (FAPP) house. All birds are housed in colony cages with 2 males and 10 to 12 hens/cage. For some specific experiments artificial insemination (AI) was used to obtain fertilized eggs. These flocks were free of CIAV infection until 1995 or 1996, when the virus was introduced most likely as a consequence of a lapse in biosecurity (7). Cardona *et al*. (2) showed that viral DNA can be present in the reproductive organs of hens and roosters independently of their antibody status. In some instances birds seroconverted between 20 and 30 weeks of age and gonadal tissues were positive for viral DNA at the end of the laying cycle. Miller *et al.* (4) examined the importance of vertical transmission of viral DNA in several experiments. Viral DNA could be detected in blastodiscs obtained from 4 antibody-negative hens, but viral DNA was only present in 50% of the eggs. Similarly, only 1 of 4 semen samples from an antibody-negative rooster was positive. Eleven roosters that were antibody-positive for at least 9 weeks prior to semen collections still produced positive samples in 18/47 (38%) of the samples, while 5 other roosters in this group were negative. Thus intermittent shedding of viral DNA through semen and eggs can occur independently of the antibody status. Examination of individual embryonal organs at 18 days of incubation indicated that viral DNA is not germ-line transmitted. In another experiment we established that eggshell membranes (ESM) were a better source for the detection of virus than pooled lymphoid organs from 18-day-old embryos. In an experiment to study vertical transmission and the feasibility to develop true negative birds we used four 64-week-old hens, which had remained antibody-negative. Each hen was inseminated with semen from an

antibody-negative, virgin male from the same flock. Hens and roosters were euthanized immediately after egg collection and the gonadal tissues were examined for viral DNA; one of the roosters had a positive testis. Eggs were placed in individual baskets, 8 chicks were hatched and immediately after hatching placed in an isolator. The ESM were tested by nested PCR for the presence of viral DNA and two chicks were eliminated because their ESM samples were positive. Four birds had positive blood samples at 6 weeks of age, one of which was also positive at 17 and another was also positive at 28 weeks of age, but all six birds remained seronegative until termination at 60 weeks of age. The next generation was obtained by mating the two birds that remained viral DNA negative and two pairs of birds that had viral DNA-positive blood at 6 weeks of age. One of these pairs produced a viral DNA-positive embryo and was eliminated. Twelve chicks were hatched from the remaining two pairs and one bird from the viral DNA-positive pair was positive at 12 weeks of age and eliminated. Eight birds were kept until 60 weeks of age and these remained negative for antibodies and viral DNA. This experiment showed that it is possible to eliminate carriers, but that this will not be an easy process (4). These experiments also demonstrated that the presence of viral DNA does not always lead to active virus replication and seroconversion. The combination of our findings led us to postulate a latency model for CIAV (7). The latency model predicts that viral DNA or virus can be transmitted vertically without leading to active viral replication unless birds are stressed for example when they become sexual mature. Viral DNA may remain present as episomal double-stranded circular DNA in gonadal tissues. Double-stranded circular DNA is required for viral replication.

We have examined the possibility that steroid hormones are involved in the regulation of viral transcription by examining the promoter/enhancer region of the virus in more detail. This region contains 4 direct repeats resembling oestrogen response element (ERE) consensus half sites, which may bind oestrogen-activated oestrogen-receptors. Transient transfection experiments using a short and a long form of the CIAV promoter were used to determine if oestrogen could enhance expression of a reporter gene. Expression of the reporter gene was indeed enhanced when we transfected the oestrogen receptor-enhanced LMH/2A cell line with the short promoter construct but not with the long promoter construct. Thus oestrogen can enhance transcription through activation of the oestrogen receptor and subsequent binding to the ERE (5). This finding is certainly compatible with the observation that SPF flocks seroconvert shortly before or after egg production has started. However to remain latent there must also be factors repressing transcription. Miller *et al.* (6) identified two repressor complexes. The first one is the chicken ovalbumin upstream promoter transcription factor 1 (COUP-TF1) which also binds to the ERE. The second repressor, transcription regulator delta EF-1 binds to a nucleotide sequence called E box-like element at the transcription start site. In conclusion transcriptional activation of viral DNA seems to be controlled by at least one positive and two negative regulators.

Serology is the standard approach to monitor the CIAV status of SPF flocks. However this may not provide a true picture of the presence or absence of CIAV viral DNA in a flock. We have recently shown that seroconversion rates can fluctuate dramatically in the closed SPF flocks at Cornell University (11). In contrast to commercial SPF flocks where facilities are depopulated at the end of the laying cycle and followed by rigorous cleaning before the introduction of the new flock, the Cornell facility is a truly closed system. Chicks are hatched in the same FAPP house where 2 to 4 flocks of adult birds are kept. Yet we have seen flocks with very low seroconversion rates after several flocks with high, but less than 100%, seroconversion rates. There are at least two explanations for these observations. The first one is that a low level of continuous horizontal infection from seropositive to seronegative birds occurs in the facility. However, this does not explain why there is a very low seroconversion rate after flocks with high seroconversion rates. The second explanation is based on the concept of latency in which the virus or episomal viral DNA is present in the gonads and can be transferred to the offspring without causing seroconversion.

The observations in SPF flocks have also consequences for primary breeders in their efforts to reduce the presence of CIAV in primary lines and grandparent flocks. Brentano *et al.* (1) and recently Hailemariam *et al.* (3) have found that viral DNA can be detected in embryos from commercial flocks independently

of the antibody status. However, it is not clear what the practical importance is in view of the widespread presence of virus in the field unless breeders would be interested in eliminating the virus from their pure lines.

In conclusion, CIAV has a well-adapted relationship with its host, in which is ensures passage to the next generation avoiding elimination by the immune responses. I believe that this virus has evolved a long time ago probably at the same time that jungle fowl evolved. In support of this hypothesis it would be of interest to test sera samples from "pure" jungle fowl that have not been in contact with backyard flocks of chickens or perhaps use historic DNA samples, if available, for analysis of viral DNA. CIAV will remain a major problem especially for the SPF industry but also for conventional poultry unless vaccines can be developed to prevent the immunosuppression associated with infection.

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CAV Vaccination, Comparison with Natural Exposure in Broilers

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Abstract:

Arbor Acres broiler breeder females were vaccinated with a commercial CAV vaccine at 18 weeks of age by intramuscular injection and others were left unvaccinated. There was no significant difference in the mean titres reached in each flock by point of lay, or during later production. The study was replicated with similar results. This finding questions the value of CAV vaccination in the face of an endemic challenge in the broiler breeder house.

Background:

Chicken Anaemia Virus is a member of the Circovirus group which was first reported as causing immunosuppression and anaemia in infected chickens by Yuasa *et al* in 1979. The virus was reported in Australia by Firth and Imai (1990). Vaccine is commercially available in Australia and is used to vaccinate broiler breeders in order to protect broilers.

This study was carried out in Victoria, Australia in an integrated broiler company in which pre-vaccination titres had been noted to be at levels generally considered acceptable in broiler breeders for protection of broilers. For examples titres of all birds in this study were over 4,000 ELISA units around 80 – 90 days of age and prior to vaccination.

Materials and methods:

Shed 1 on the parent rearer farm contained 11,900 eighteen week old Arbor Acre broiler breeders and was treated with Intervet Nobilis CAV P4 vaccine (Batch number: 3949-007) by intramuscular injection on the 12th of August 2009.

A one thousand dose vial of CAV vaccine was injected into a 500ml bottle (1,000 doses) of Nobilis EDS + ND Combined inactivated vaccine against EDS 76 and Newcastle Disease (Intervet Australia Pty Ltd). This was injected into the breast muscle of each bird at a dose rate of 0.5 mL Shed 2 on the same farm also was stocked with eighteen week old Arbor Acre broiler breeders and was left untreated.

The study was replicated in sheds 3 and 4 on the same farm with the same bird numbers in shed 3 and 12,000 in shed 4. Birds in shed 4 were vaccinated at 18 weeks with the same batch of vaccine on the 23rd of September 2009.

Ten serum samples were collected from each group on several occasions up to the age of 53 and 47 weeks and CAV ELISA testing (BioChek Lot Numbers fs4869 & fs4982) was performed by Ace Laboratory Services (Lot 3, Gildea Lane, Bendigo East, Vic 3550).

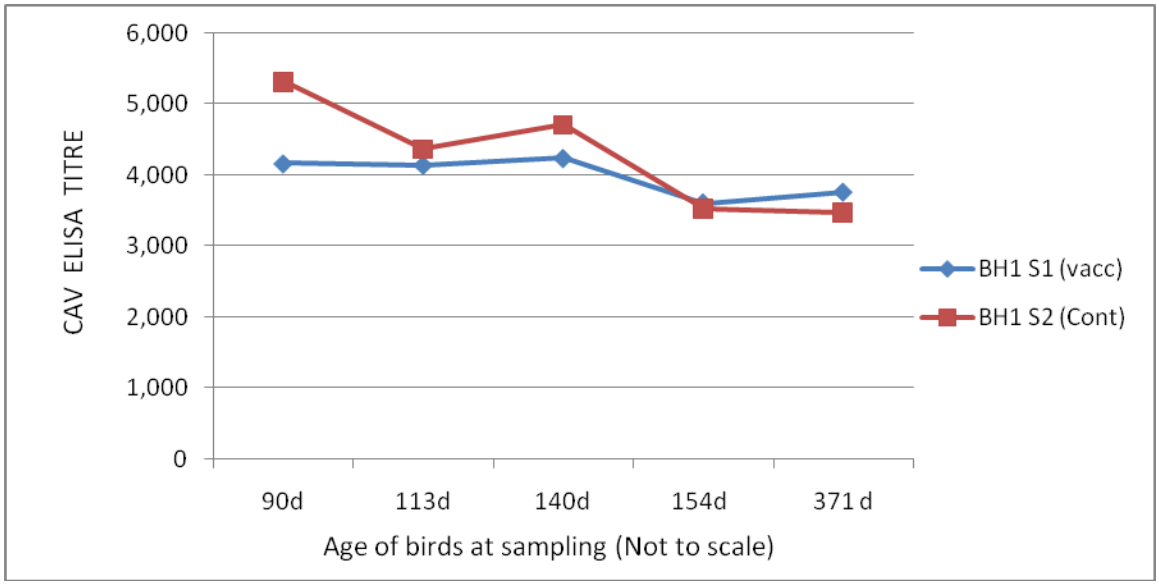
Results and discussion:

Results from the two replicates are summarised below in graphs1 and 2.

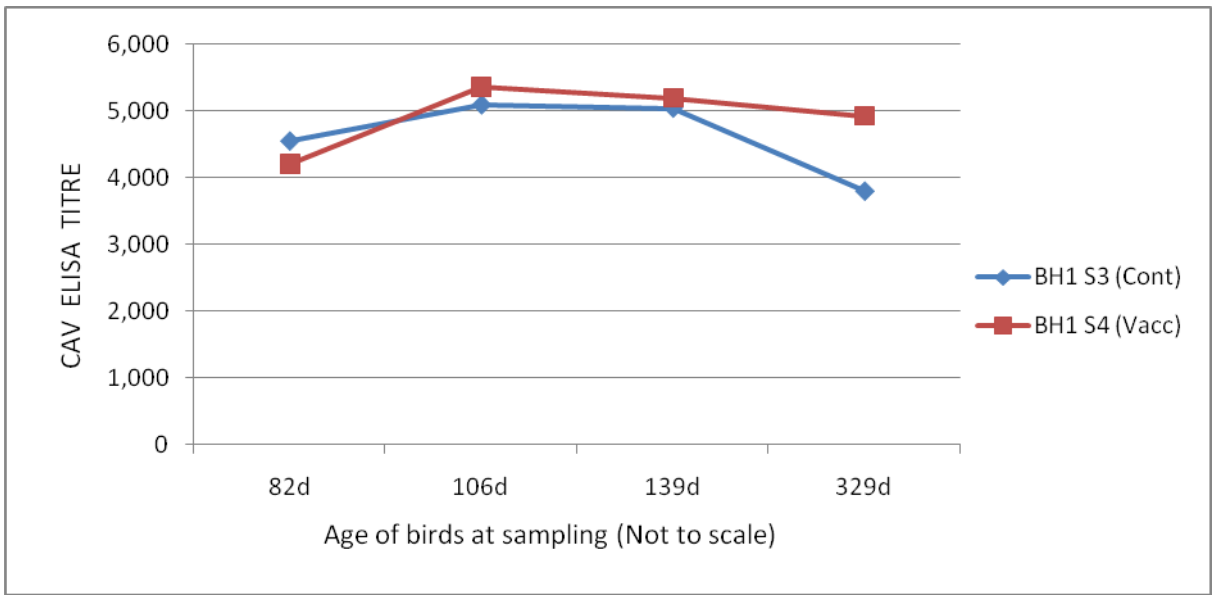
Birds were vaccinated at 18 weeks of age (approx 126d) and every bird tested was positive (over 725 ELISA units) at all time points. The serology indicates that all birds had been exposed to CAV prior to vaccination and no major serological response to vaccination was observed.

A trend towards a significant difference ($P > 0.13$ - two tailed Students t-Test) between vaccinates (mean ELISA 4,928) and controls (mean ELISA 3,802) at 47 weeks of age in sheds 3 and 4 was observed. This was not seen in the first replicate of birds (sheds 1 & 2) at 53 weeks of age.

Graph1: CAV ELISA titres in broiler breeders from with or without vaccination



Graph 2: CAV ELISA titres in broiler breeders from with or without vaccination



The study lends some weight to the following hypothesis; in the operation where the study was conducted, broiler breeders will be exposed to CAV in rear and develop sufficient immunity to enable protection of chicks by passive transfer of antibodies.

It is not recommended that broiler breeder operations cease CAV vaccination in the absence of a history of adequate antibodies prior to the normal time of vaccination and further serological monitoring post vaccination.

Statistics

A Student's t-Test* was used to examine the difference between the 47 week serology of sheds 3 and 4.

t-Test: Two-Sample Assuming Unequal Variances

	BH1 S3	*BH1 S4*
Mean	3,801.6	4,927.9
Variance	2,133,688	2,911,958
Observations	10	10
Hypothesized Mean Difference	0	
df	18	
t Stat	-1.58561	
P(T<=t) one-tail	0.06512	
t Critical one-tail	1.734064	
P(T<=t) two-tail	0.130239	
t Critical two-tail	2.100922	

- Microsoft Office Excel 2007 Microsoft Corp

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Case Report: Avian Nephritis Virus (ANV) Infection in a Broiler Flock in Victoria

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Baiada Poultry

Back in June 2009, a flock of broilers in western Victoria exhibited an unusually high early mortality around the second week of age, starting from one shed and moving rapidly across to all four sheds. The affected birds look depressed, below target weight and an unmistakable respiratory snick/sneeze was present. Initial significant post-mortem findings revealed severe swelling of the kidneys and ureters in all birds examined. Infectious bronchitis (IB) infection was first suspected, as a possible vaccination reaction but the clinical picture-morbidity, mortality and severity of renal damage was too difficult to reconcile with an IB infection at this age with no other broiler farms exhibiting the same problem in spite of the same vaccination procedure used in the hatchery.

Kidney samples sent to Melbourne University initially picked up the IB vaccine strain. However, further testing (PCR) and virus isolation yielded the astrovirus-ANV. This is one of the first few isolations of this virus in Australia.

It is also surmised that very early infection of ANV can possibly cause immunosuppression manifesting as increased susceptibility to infection from the "normal" flora found inside the shed.

The rest of the presentation discusses the effect of ANV on performance parameters both in this particular affected flock and the subsequent flock followed by the procedures undertaken to control this emerging infection.

Isolation of Avian Nephritis Virus from Australian Chicken Flocks

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Introduction

Avian nephritis virus (ANV) is an avian Astrovirus that causes significant disease in poultry aged up to 14 days old (2, 4, 7, 8) and is associated with severe interstitial nephritis. Infection with ANV results in growth retardation of the affected poultry and therefore has negative economic implications. ANV has been reported in poultry exhibiting clinical signs of renal distress, with similar signs observed after experimental infection, but has also been repeatedly detected in apparently 'healthy' flocks, including specific pathogen free flocks (1, 2, 5-7, 9, 10).

ANV has very recently been detected in Australian chicken flocks in NSW and VIC (3). To allow further investigation into ANV in Australia, isolation of the virus is required.

Results

Swabs and / or kidney tissue were collected from 8-11 day old (d.o.) broiler chicken flocks in New South Wales and Victoria regardless of the current or previous health status of the flock.

The CPE observed in the CEK cells was distinct from known Australian avian pathogens and embryos from the CAM inoculated eggs showed no pathology other than a slight dwarfing when compared to non-inoculated controls. Gross examination of the CAMs for all six submissions showed the same lesion, but of varying sizes, ranging from 10% to 70% of the inoculated area. Histopathological examination of infected CAMs showed massive ulcerative and necrotic lesions.

Common practice for examination of CEK cells inoculated with an unknown avian pathogen is to observe, and take initial samples at, 24 hours p.i. Due to the lack of a live characterised ANV to use as a positive control in our laboratory, PCR was used for confirmation of viral growth. A time trial experiment, using submitted renal tissue inoculated onto CEK cells, was designed to determine if there was an optimal time point for detection of ANV by PCR in CEK cells.

An ANV positive band was produced from the inoculated CEK cells at 2 – 4 hour p.i. only. This band was subjected to nucleotide sequencing and matched the nucleotide sequence of the ANV originally detected in the kidney tissue.

Discussion

There is currently no characterised live Australian ANV available for comparison against suspected ANV submissions. In addition, there is no commercial serological test currently available. Thus PCR and nucleotide sequencing was used to confirm that ANV had grown in CEK cells.

Our laboratory has extensive experience with the culture of a wide range of avian viruses and the CPE generated by them. However, the CPE found in association with ANV growth in CEK cells and the lesions observed on the CAMs was dissimilar to all those previously seen in our laboratory. It is very likely that other, currently uncharacterised, viruses that infect chicken kidneys were also present in the original tissue submission.

The successful isolation of an Australian ANV lays the foundation for molecular and serological identification of the Australian ANVs.

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A Survey of the Occurrence of "Spotty Liver Syndrome" in Commercial Layer Chickens

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Introduction

The so called "Spotty Liver Syndrome" (SLS) or Miliary Focal Hepatitis has been a problem in adult female hens (meat and layer strain breeders and commercial layers) that are kept on the floor for many years. This has been particularly so for free range and barn style commercial egg producers. The syndrome appears as an acute hepatitis involving a sudden onset of mortality and decreased egg production mainly seen in flocks in early lay in the warmer months of the year. Culture and histological analysis of the livers of these birds has not shown the presence of a definitive causal bacteria yet the condition responds rapidly to antibiotic medication. Several attempts at defining the causative agent have failed in the recent past and the aetiology remains elusive and is in all expectations multifactorial.

The epidemiology of the syndrome is poorly understood and comprehensive field observational studies are lacking or nonexistent. To this end, we conducted a simple mail survey of the free range and barn style egg producers in NSW and Victoria over the 2008-2009 summer period. Results are presented.

Materials and Methods

A simple questionnaire was mailed to all identifiable free range and barn layer operations within NSW and Victoria (a total of 45 farms), asking the farms to report on a flock of layers placed in their operation between October and February.

The questionnaire requested information on bird age, breed, density, rearing location, barn or free range style, vegetation in range, litter type, nest type, feed source, worming program, occurrence of SLS, who provided the diagnosis, any treatment given and its effect and any other flock problems (wet litter, cannibalism, respiratory disease, other).

Results

23 farms responded to the survey (18 NSW, 3 Vic, and 2 Qld) which was a response rate of 51%. Several responded saying that they no longer farm birds. Seven farms reported SLS over the study period (30% of respondents). Five cases were "diagnosed" by a serviceman, only 2 by veterinarians. The age of onset varied between 25 and 30 weeks. All cases were medicated. Two cases reported recurrence within a few weeks, requiring follow up treatment.

Losses in case farms varied between 16 and 150 birds (0.5 to 1.0%) prior to treatment. Egg production dropped between 0.1% and 15% (this was not proportional to bird losses).

No significant associations could be defined between the occurrence of SLS and breed (P=0.34), vegetation cover of range area (P=1.00), wet litter (P=1.00), mash versus pelleted feed (P=0.26), home mix versus commercial mill feed (P= 0.33), automatic nests (P= 0.25), occurrence of worms (P=1.00), use of a worming program (P= 0.37), occurrence of cannibalism (P=1.00) or prolapses (P=0.25) or bird stocking density (P= 0.71).

On univariate preliminary analysis there were some factors showing an interesting association with the occurrence of SLS, namely birds reared in Victoria (P= 0.02), absence of litter in the scratch area (P= 0.17), town water source

(P= 0.16) and the number of birds placed (P= 0.006). When a multivariate analysis was applied to control for the presence of all these factors, only flock size remained significant. Case flocks averaged 13,683 birds while non case flocks averaged only 7,003. Flocks with more than 12,000 birds appeared to be more at risk and this was not associated with stocking density.

Discussion

This was a simple survey and the results may be subject to considerable confounding. The association with flock size however was considerably strong. Flock size on its own may be a surrogate risk factor for another underlying factor which is strongly correlated to it but was not measured by the information gathered. It is hypothesised that the real risk factor may relate to actual available feed space. Many flocks have slatted areas adjoining the nest boxes and drinkers and waterers are placed over this slat area to encourage birds to use the nests. In some cases, the feed lines are very close to the edge of the slats. It is hypothesised that in hot weather when the birds are approaching peak egg production and are at their hungriest, that birds leaving the nest boxes and crowding to the feeders may push the feed lines close to the edge of the slatted area, thus decreasing real feed space availability. This "stress" may be the factor which predisposes the flock to the occurrence of SLS. This possibility has considerable anecdotal support (R. Horn, pers. comm., C. Owens, pers. comm.).

This hypothesis needs to be investigated by a more thorough study focussing only on flocks above 12,000 birds during a summer period. If confirmed, this would provide a tool to control or eliminate this disease from the industry without the need to discover the identity of the causative organism(s) involved.

Acknowledgements

The author wishes to thank all the layer farmers who contributed to the survey and to Rowly Horn, Joe Wegryzn, and Col Owens for their encouragement of the farmers to cooperate in the survey.

Embryology Explained

Donna Hill

Preliminary Trends from a Study on Food-Borne Pathogens and Litter Re-use across Broiler Cycles

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Introduction

Re-use of litter will, in the future, be a key issue particularly availability and quality of suitable bedding material. Currently re-use is practiced in Queensland as an alternative to sourcing new bedding for each cycle. Re-use will also address issues around the disposal of used litter to the environment. However there is an absence of scientific data to support the re-use of litter. This lack of data has lead to concerns from time to time on the possible transfer of pathogens from cycle to cycle via used litter or even a build up in pathogen levels following sequential production cycles. However in countries such as the USA, full re-use of litter does commonly occur for an extended period of time.

The national data for gastrointestinal illnesses in Australia in 2009 list Campylobacteriosis (15,841 cases) and Salmonellosis (9,523 cases) as the two major causes of gastrointestinal illness (Australian Government, Department of Health and Ageing). This creates pressure on the poultry industry as both organisms are normal inhabitants of poultry. Knowledge of the effects on both *Campylobacter* and *Salmonella* levels when litter is re-used would create better understanding of the impact of commonly adopted re-use practices and the survival potential of these key pathogens.

The recently completed CRC project (Re-use of chicken litter across broiler cycles – managing the food-borne pathogen risk, 2006-2008) and the current RIRDC project (Evaluating food-borne pathogen transfer associated with partial and full re-use litter 2008-2011) were both designed to address the relationships of both *Salmonella* and *Campylobacter* during re-use. The CRC study dealt with litter treatment (i.e. litter windrowing/piling) between cycles and the RIRDC study (in progress) deals with the impact of re-used litter on pathogen levels in both the chicken caeca as well in litter used through the different production cycles.

In the current RIRDC project, we are working on understanding the dynamics of these two pathogens during three different litter management practices, i.e. fresh bedding each cycle, a common Australian practice (partial re-use) and full re-use. One shed has been dedicated to each litter management practice. The current study deals with comparing these three production practices on a single farm under commercial conditions. The full study is looking at possible pathogen transfer across cycles via the litter and is designed to test six sequential cycles with re-use litter (both full and partial) being used for almost a year. The shed operating conditions as well as key physical parameters (Moisture and pH) of litter are also being monitored during production.

This study is still in progress. Initial outcomes suggest there is little difference across the three sheds (levels in litter and levels in caecum) through sequential cycles even with the use of varying litter practices. An overview of these outcomes will be discussed. At this early stage, there appears to be a potential to re-use litter based on either practices of re-use being studied.

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Acknowledgements

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The support of Kelly McTavish (Ingham's Enterprises) during the trials is gratefully acknowledged.

The support of RIRDC for the funding provided for the current work is gratefully acknowledged. The support of the Poultry CRC for funding provided for the past work is gratefully acknowledged. The support of the farmers who have participated in past trials and contributed to the build-up of knowledge on litter re-use in is gratefully acknowledged.

Towards Improved Inactivation and Monitoring of Viral Pathogens in Reused Broiler Litter

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Introduction

Re-use of litter by broiler chickens can reduce the cost and environmental impact of production but uptake of the practice is limited largely by the risk of pathogen carryover between batches. On a recent project funded by the Australian Poultry CRC (Project 06-15) our research team developed an effective chick bioassay to measure viral infectivity in litter (1). We also conducted field experiments investigating the effects of various litter heaping treatments on decay of viral infectivity over 9-10 days (2) and associated temperature, pH, moisture and litter chemistry measurements (3,4).

One objective of this work was to link the temperatures and other changes achieved during litter treatments to the rate of pathogen inactivation as determined by bioassay of litter infectivity. Ideally such information would be generated for the complete range of viral pathogens, but a problem with the approach taken was that sometimes there was a limited range of pathogens on a given farm in question. One solution would be to experimentally infect chickens and thus litter with a wide range of pathogens, but it is difficult and expensive to do this on scale which would generate sufficient litter for composting studies that would mimic the field situation. Another problem with wider application of methodology to measure viral pathogen load in litter is the comparatively high cost of the chick bioassay.

Methods, results and discussion

In 2009 the Poultry CRC funded a short project (09-34) to test proof of concept of approaches to overcoming the issues raised above. To better determine temperature/time requirements for virus inactivation we deliberately generated infective litter with vaccinal strains of the major viral pathogens, then exposed this litter, together with "dirty" end of batch field litter to temperatures of 50, 60 or 70˚C in incubators for 10 days. Samples taken on days 0 (before heating), 5 and 10 of these treatments were tested for the presence of viral pathogens using the chick bioassay. To examine the possibility of molecular detection of viral pathogen load directly from litter, we undertook preliminary investigations into molecular quantification of MDV in litter material. Results are summarised below.

Production of infective litter and transmission of pathogens in day 0 litter (prior to heat treatments).

Forty commercial broiler chickens were used as shedder birds to produce contaminated litter. Between days 17 and 28 they were infected with vaccinal strains of IBDV, NDV, IBV, MDV, CAV (old Steggles vaccine), Fowl pox virus, ILTV and AEV. At day 35, when litter was collected for treatment and testing, birds were serologically positive for CAV, MDV, ND, IBD, IB and ILT although the response to the latter was low. The low sero-conversion rate for ILT was probably because blood samples were collected only 8 days after vaccination. The chickens were not positive for AE antibody possibly because serum was collected only 7 days after vaccination. Seroconversion to fowl pox was not measured,

as we did not have an assay for it. Litter from the UNE shedder chicks resulted in successful litter transmission of MDV and CAV only. There was no litter transmission of IBV, NDV, IBDV, ILTV or AE from these chicks despite the shedder chicks being seropositive at the time of litter collection. This may be due to lack of shedding of virus (wrong window of time, low shedding rates) or failure of the virus to transmit on litter. We know that IBV and NDV transmit poorly on litter (Islam et al., 2010a) but that IBDV will, and in this experiment the field litter was also sporadically infectious for IBDV. This suggests that our model for generating infectious litter requires refinement and measurement of actual viral shedding in addition to seroconversion.

The pooled field litter contained highly infectious FAV, for which we did not have challenge virus at UNE. It was also infectious for MDV, CAV and IBDV (sporadically).

Virus inactivation over time at different temperatures.

FAV infectivity was lost by day 5 at all temperatures (50, 60 and 70 °C). CAV infectivity was lost by day 5 at 60 and 70˚C and by day 10 at 50˚C. Data for IBDV (in the field litter) were inconclusive, however there was no infectivity detected by day 10. However significant MDV infectivity was maintained at day 10 for all temperatures for the field litter, and at 50˚C for the UNE litter. Temperature effects could not be tested for IBV, NDV and ILT as there was no apparent litter transmission at all.

Detection and quantification of viral DNA directly from litter.

We were able to directly measure MDV viral load in litter using a simple protocol on our 1st attempt. However there was a poor association with the level of infectivity determined by the chick bioassay. Due to strange pattern of MDV infectivity in this experiment and because methods of viral quantification from litter had not been optimised we feel that this approach is worthy of further investigation on a wider range of viruses with clearer patterns of inactivation.

Conclusions

In broad terms proof of concept was confirmed for:

- Laboratory level heat treatment of litter, coupled with bioassay of infectivity, to better define temperature/time interaction for inactivation of pathogens in litter;
- direct detection and quantification of viral pathogens in litter.

This offers promise for a future situation where infective viral pathogen load in litter is measured directly and the effects of between batch litter treatments on viral inactivation are readily determined by direct methods. Nevertheless significant challenges remain before this becomes a reality. These include:

- Optimising chick challenge methods to guarantee infective litter during the experimental phase;
- Optimising methods of extracting and quantifying viral nucleic acids from litter;
- Defining the relationship between decay in molecular detection and decay in infectivity for different pathogens. The two may not necessarily coincide (5).

Acknowledgements

We are grateful to the Australian Poultry CRC for funding this work under Project 09-34. We thank Gary Taylor for assistance with the isolator experiments and Maurice Velchic and Phil Ashby for their assistance in supplying field litter.

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Identification of Chlamydial Species in Chickens by PCR-HRM Curve Analysis

T. Robertson, S. Bibby, D. O'Rourke, T. Belfiore, R. Agnew-Crumpton and A. H. Noormohammadi

Recently, a PCR protocol (16SG), targeting 16S rRNA gene coupled with high resolution melt (HRM) curve analysis was developed in our laboratory and shown to reliably detect and identify the seven different *Chlamydiaceae spp*. In this study, the potential of this method was assessed for detection and differentiation of Chlamydiosis in clinical specimens. Of the total number of 733 specimens from a range of animal species, 199 (27%) were found positive by 16SG PCR. These included 27 positive specimens from a total number of 306 chicken specimens. When a sufficient amount of DNA was available (9 chicken submissions), amplicons generated by the 16SG PCR were subjected to HRM curve analysis and results were compared to that of nucleotide sequencing. Analysis of the HRM curves and nucleotide sequences from 16SG PCR amplicons revealed the occurrence of a *Chlamydophila*-like, a *Parachlamydia*-like and a variant of *Chlamydophila psittaci* in chickens. These results reveal the potential of 16SG PCR-HRM curve analysis for rapid and simultaneous detection and identification of *Chlamydiaceae spp*. in chickens.