AUSTRALASIAN VETERINARY POULTRY ASSOCIATION

SINCE 1961

PROCEEDINGS – SCIENTIFIC MEETING

11TH & 12TH FEBRUARY 2021

Zoom Video Conference

AVA | COMMERCIAL POULTRY VETERINARIANS

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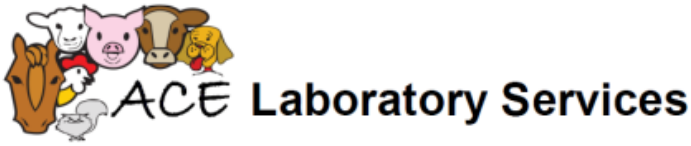

AVPA Scientific Program
Zoom – 11TH to 12TH February 2021

THURSDAY 11th FEBRUARY *Australia Eastern Daylight Time Zoom		
Time	**Speaker**	**Topic**
9.00-9.10	**Sheridan Alfirevich, President AVPA**	**OPENING**
	Chairperson: Peter Gray	**Avian Influenza Outbreak, Victoria**
Sponsors	**BEC** **Jefo**	**Nutriment Health** **Zoetis**
9.10-9.30	Initial Findings- A Poultry Practitioner's View	Dr Peter Scott, Scolexia
9.30-10.00	A Government View	Dr Megan Scott, DPI Victoria
10.00-10.15	An Industry Liaison Officer View	Dr Bany Oyay, Turi Foods
10.15-10.30	Private Practitioner View	Dr Rowan Wilson, Nutriment Health
10.30-10.45	Panel Discussion	
10.45-11.15	**Morning Tea**	
	Chairperson: Fabian Barcelo	**What in the World**
Sponsors	**Alltech Lienert** **Bioproperties**	**Poultry Hub**
11.15-11.45	Current Turkey Disease Internationally	Dr Dustin Burch, Aviagen Turkeys Inc., US
11.45-12.05	Current Turkey Disease in Australia	Dr Sheridan Alfirevich, Baiada
12.05-12.15	Panel Discussion	
12.15-12.45	Current Duck Disease Internationally	Dr Alice Ghibaudo, Grimaud Frères
12.45-13.05	Current Duck Disease in Australia	Dr Karen Gao, Zootechny
13.05-13.15	Panel Discussion	
13.15-14.00	**Lunch**	
	Chairperson: Sheridan Alfirevich	**ILT SA – A Success Story**
Sponsors	**AgriFutures Chicken Meat** **EW Nutrition** **Lallemand** **Treidlia BioVet**	
14.00-14.30	ILT Control in SA	Dr Margaret Sexton, PIRSA
14.30-14.50	Practical lab techniques to assist field control of ILT	Dr Steve Walkden- Brown, UNE
14.50-15.00	Panel Discussion	
15.00-15.20	**Afternoon Tea**	
	Chairperson: Sheridan Alfirevich	**Epidemiology**
Sponsors	**ACE Laboratory Services** **Biomin**	**MSD**
15.20-15.40	Interpretation of Field Epidemiological Studies- advantages and hazards	Dr Peter Groves, Zootechny
15.40-16.00	Spotty Liver Disease Epidemiology	Dr Karen Gao, University of Sydney
16.00-16.10	Panel Discussion	
16.10-16.15	**Final Wrap-up**	

AVA | COMMERCIAL POULTRY VETERINARIANS

AUSTRALASIAN VETERINARY POULTRY ASSOCIATION

AVPA Scientific Program
Zoom – 11TH to 12TH February 2021

FRIDAY 12st FEBRUARY **Australia Eastern Daylight Time* ***Zoom***		
Time	**Speaker**	**Topic**
	Chairperson: David Sherwood	**Poultry Pathogens**
Sponsors	**BASF**	**CCD Animal Health** **Elanco**
8.30-8.50	The impact of toxigenic moulds and mycotoxins on poultry health	Prof Johanna Fink-Gremmels, Utrecht University
8.50-9.10	Salmonella- longitudinal survey and use of probiotics	Dr Kapil Chousalkar, University of Adelaide
9.10-9.30	Seasonal variation of key food safety pathogens in the processing plant	Jillian Templeton, DAF Qld
9.30-9.50	Fowl cholera- genomic analysis to investigate outbreak dynamics	Dr Lida Omeleki, University of Queensland
9.50-10.00	Panel Discussion	
10.00-10.15	**Morning Tea**	
	Chairperson: Christine Clark	**New technologies**
Sponsors	**FeedWorks**	**PacificVet** **Premium Agri Products**
10.15-10.35	Video analysis of flock motion in commercial sheds for health prediction	Dr Cheryl McCarthy, University of Southern Queensland
10.35-10.55	Welfare aspects of HatchCare	Dr Ashley Etherington, Ingham's Enterprises
10.55-11.05	Panel Discussion	
11.05-11.10	**Closing**	
11.10-11.20	**Short Break**	
11.20-12.20	**AVPA AGM**	

Avian Influenza Outbreak, Victoria
Initial Findings – A Poultry Practitioner's View

Dr. Peter C. Scott

Scolexia Animal and Avian Health

The key principles regarding any Emergency Animal Disease (EAD) are its prompt recognition and diagnosis, containment, eradication and finally proof of freedom. The later being important to allow industry to get back to normal operations regarding domestic movements and international trade.

This report covers the High Path Avian Influenza (HPAI) H7N7 outbreaks in commercial layers and not the Low Path AI (LPAI) H5N2 in turkeys or the LPAI H7N6 in emus which were concurrent outbreaks in Victoria at the same time.

The Infected Property 1 (IP1) in Victoria was located in the Golden Plains shire being a relatively new free-range operation consisting of two naturally vented flat deck sheds each containing approximately 22,000 birds. The property was of a high husbandry and biosecurity standard, understanding the limitations of a free-range operation, with boundary amenities, secure fencing, shower on facilities and serviced by mains town water supply and secure feed deliveries and storage. Eggs were collected several times weekly and taken to a centrally located grading floor which received eggs from multiple farms, both company and contractor, but all under the control of the one entity. The IP1 egg truck serviced only contractor farms which were located in the North West and North Central parts of Victoria. The egg truck was cleaned and disinfected between runs, eggs were stored at the grading floor with clear identification of the farm source and egg fillers and modules were disinfected ongoing.

The high-risk aspects of the farm were those experienced by most free-range operations being the mandated planning requirement of a retention dam and the observation of wild waterfowl (including ducks and swans) being not uncommonly observed on the site.

The atypical aspect of this first outbreak of HPAI H7N7 was the rapidity of onset of the clinical signs and mortality, with the normally observed clinical findings of the involvement of LPAI progressing to HPAI, not evident. The production in both sheds at 52 weeks of age was 92% hen day. Shed 1 at 52 weeks of age had a weekly mortality (WE 26-07-21) of 6 birds and accumulated mortality of 1.68% and between the 27-29 July only a further 2 mortalities. Shed 2 at 52 weeks of age had a weekly mortality (WE 26-07-21) of 6 birds and accumulated mortality of 1.36%, but for the 3 days including 27, 28 and 29 July, the mortalities were respectively 4, 69 and 169, with HH production still being 90% on the 27th July, but declining to 82% on the 28th July and prompting the visitation. The farm was visited AM on Wednesday 29th and quarantined, samples collected and submitted to AgriBio, Agriculture

Victoria, with confirmation of AI at 8 PM that night. It was the history of this site being of such a high health status and free of diseases like fowl cholera, and the presenting signs of a high level of severe depression, increasing mortality, a significant production drop and diarrhoea, that resulted in the immediate suspicion of an EAD.

IP2 was diagnosed approximately one week later with AI and subsequently confirmed as H7N7. This free-range site consisted of 3 aviary sheds of approximately 30,000 birds each. There had been leading up to the diagnosis of AI on IP2 some investigations around performance associated with low peaking performance in one shed. The notification by the farm manger to technical services of clinical signs identical to IP1 prompted an immediate investigation and sampling by Agriculture Victoria. While IP1 is located approximately 6 kilometres from IP2, there were no horizontal contacts of any type recognised between the two sites supporting independent infections and highly likely to be wild waterfowl as the source. IP2 was in close proximity at approximately 1 kilometre to IP3, a large cage layer complex owned by the same entity of IP2. Epidemiologically it was identified that while no clinical signs were overtly initially evident on IP3, testing at the same time as the testing of IP2 revealed IP3 was AI positive. There were a number of horizontal contacts between the two sites involving farming, maintenance staff and egg cartage vehicles.

IP4 a free-range farm of 3 converted broiler sheds and contracted to the entity of IP2 and 3 also became AI positive approximately two weeks later despite the birds being confined at the time of diagnosis of IP2 and 3. While the source of infection is unclear a preceding infection from wild birds noting, there are multiple dams on the site in close proximity to the sheds, or horizontal contacts with the operational aspects of IP2 and 3 are the most probable. The concern about further spread through horizontal contacts initiated the quarantining of all the In Contact (IC) farms until site investigation regarding clinical signs, mortality figures and testing by PCR over several weeks proved freedom from AI: an anxious period for farm owners.

Overall, the response times of recognition, diagnosis and containment were satisfactory. There was evidence that the horizontal contacts associated with the operations of the affected farms are high risk activities but there are fundamental logistical and commercial aspects that limit the avoidance of these. Utilisation of eggs trucks visiting multiple sites is the major one and often a number of sites during the one run collecting eggs and dropping off replacement fillers and modules. This was identified as source of spread in the NSW, Young outbreak.

The responsible authorities within Agriculture Victoria introduced a confinement mandate for all free-range birds in the Golden Plain shire due to the concern about further spread of AI through contact of commercial poultry with wild waterfowl. The anomalous consideration here is that after removal of the confinement notice there is return of this risk which will again be dependent of the AI sylvatic cycle in wild waterfowl.

Going forward, with the increasing number of birds farmed under free range, there is a need for a pragmatic understanding by regulatory authorities and legislators of the AI risks that are being imposed on producers due to planning obligations to have retention dams on sites and the inability to be allowed to control waterfowl on site by the issue of permits to allow limited shooting of waterfowl as a form of

aversion. Other aversion methods are limited in consistently controlling wild waterfowl and the use of dam mitigation facilitation does not impede the grazing of waterfowl on the range.

The cost of outbreaks, the withdrawal of the chicken meat industry from funding compensation under the EAD Cost Sharing Agreement of AI outbreaks in egg layers and the concerns of financiers and insurance companies, obligates reassessment of the free-range industry by regulators. If not, we will follow the path of the current AI scenario in Europe and the UK. It is important to be proactive in regard to AI and not reactive.

Avian Influenza Outbreak, Victoria

A Government view

Dr Megan Scott

Chief Veterinary Officers Unit, Agriculture Victoria

In July and August 2020 three different avian influenza serotypes were detected across three distinct geographical regions in Victoria. High pathogenic avian influenza (H7N7) was confirmed at four properties near Lethbridge, low pathogenic avian influenza (H5N2) at Lethbridge and near Bairnsdale and low pathogenic avian influenza (H7N6) at an emu farm near Kerang.

Agriculture Victoria has a legislated responsibility to coordinate and respond to incidents associated with emergency animal diseases such as avian influenza (H5/H7 serotypes).

This presentation provides an overview of the outbreaks, response actions and current situation from the perspective of Agriculture Victoria and is intended to compliment other presentations in this session from other response stakeholders.

Avian Influenza Outbreak, Victoria
An Industry Liaison Officer View

Dr Banydhuro Samson Oyay

Poultry Veterinarian, Turosi Food Solutions Group

Beginning on the 31th of July 2020, Victoria, Australia suffered a series of Avian Influenza outbreaks within a time frame of just over 3 weeks. THREE different strains of avian Influenza, namely H7N7 highly pathogenic avian influenza (HPAI) in hens at Lethbridge area, H5N2 low pathogenic avian influenza in turkeys at Lethbridge area and later in East Gippsland, and H7N6 low pathogenic avian influenza (LPAI) in Emus at Kerang, North West of Victoria.

The mechanisms of emergency disease response stipulated in the AUSVET plan were enacted and still being executed at the time of writing this abstract in a timely manner. Eradication was the recommended and approved response plan by CCEAD and NMG respectively. The response has been quite efficient in containing the outbreaks to their original localities except for an unsolicited movement of turkeys from an infected premises to a remote farm location within the Victorian state. The moved turkeys later tested positive to the same low pathogenic strain of AI. Such actions highlighted the need for tailored biosecurity education at different levels for players in poultry industry.

Challenges included the choice of available methods for humane euthanasia of infected premises and Biosecurity considerate disposal of carcasses. Whilst water based foam proved effective on barn type housings and particularly when applied to relatively healthy birds, this process can be significantly slowed when applied to lethargic birds. Therefore, CO2 destruction methods came handy in finishing the job with caged type housings. Further, in some situations, the option for local burial of carcasses was hampered by environment considerations and soil unsuitability for excavation work. This created an added burden in risk management for offsite disposal at the closest landfill. The response also experienced under resourcing in veterinary staff in the early stages. This was later fixed with AVA assistance.

Despite the successful conduction of the response, there are lessons to be learned from this outbreak as per my experience as an Industry Liaison Officer. I may summarise these observations in the following outlines:

- There is need to improve the relationship between the industry and the government to that of serious partnership. I trust a higher trust level and active involvement in decision making would add much needed value to the response outcome.
- Effective and timely communication is essential to avoid third party interference with official flow of information to stakeholders, producers and the public.
- AUSVET guidelines produced in the context of emergency animal disease can be improved upon, helping to assure the trustworthiness and utility of Livestock liaison Officer Expertise and connections for future emergencies.

- The principles of transparency, minimization of the risk of bias, and reliance on research and other evidence over expert opinion, apply to guidelines developed in any setting or context.
- With careful self-evaluation, thoughtful reflection on lessons learned in these emergencies, and a firm commitment to continuous improvement, ACMF may continue to provide timely and high-quality guidance in the context of POULTRY health emergencies, i.e. Training.
- Investment in disposal facilities such as rendering machines may assist in avoiding risky movement of infected materials for burial outside the infected premises.
- Proper resourcing with particular emphasis on veterinary technical expertise may save time and fast tract response progress.

Avian influenza 2020: A practitioner's view

Dr Rowan Wilson BVSc MANZCVS

Technical Services Manager, Nutriment Health Pty Ltd

During the Avian influenza outbreak in Victoria in 2020, a call went out to private veterinarians to assist the Victorian Department of Agriculture with the response. I was one of about 30 vets who responded to this call and were employed by the department to help.

The initial call for assistance came via the AVA, who had been involved with negotiations with government departments about the employment of private vets in government responses back as far as 2008. Whilst this call did go to many practitioners, it did not reach some members of the AVPA who were not AVA members, so some vets with poultry expertise were not aware of the call.

The "on boarding" process went smoothly for most people. All respondents needed to have Q fever and seasonal influenza vaccinations as non-negotiable conditions of employment. Other required paperwork included police checks, an EMMQ questionnaire which covered one's physical and mental health, and the usual tax and superannuation forms. In my case the entire employment process took less than a week.

Vets were initially employed for 5 day rotations based in the Local Control Centre at Ballarat. Accommodation and all meals were supplied, with local motels and hotels no doubt appreciating the business in the COVID lockdown conditions that were prevailing.

Private vets were not involved with any of the infected premises at any point. Our role was within the surveillance team and consisted of visiting properties in the restricted and controlled zones. This included some large commercial premises but also many small properties with backyard poultry. Birds on selected farms were generally sampled with choanal and cloacal swabs which were couriered to the lab on a daily basis.

Full PPE was used to enter farms on all occasions, and I found that performing the entry/exit procedures multiple times gave me an important take-home skill which could be useful in any future exotic disease outbreak.

Other duties included organising faecal sample pickups from farms, collecting dead commercial and wild birds, and helping with the initial processing of lab samples.

As the response continued, we found that the 72 hour down time after visiting farms was inefficient, so the rosters were changed so that most of us were employed for two separate days per week, in my case Monday and Thursday. This worked well, especially for practitioners living within two hours of the response area.

Overall, I think the employment of private vets made a positive contribution to the response, and this should be considered in any future outbreak. It may be worth seeking expressions of interest from vets who may be willing to help in any future response, and perhaps a database kept so they may be rapidly contacted and deployed when needed.

Current Turkey Diseases Internationally

Dustin B. Burch, DVM, MPH, MAHM

Aviagen Turkeys, Inc.

In the turkey industry today there are many diseases that have and do commonly occur worldwide. These diseases in return cost the U.S. turkey industry alone hundreds of millions of dollars' worth of lost revenue due to mortality, increased FCR, condemns at processing, and increased labour resulting in higher overall production costs. In this presentation I have chosen ten of the more common turkey diseases seen in the United States and internationally to discuss routes of transmission, clinical presentation with lesions noted, means of diagnosis, treatment, and control/prevention for each disease. The diseases presented here will be *Salmonella* (Paratyphoid), Colibacillosis (*E. coli* infection), Fowl Cholera (*Pasteurella multocida*), *Ornithobacteriosis rhinotracheale* (ORT), Erysipelas (*Erysipelothrix rhusiopathiae*), Dermatitis (Gangrenous & Necrotic), Necrotic Enteritis, Coccidiosis, Histomoniasis (Blackhead), and Reovirus (Viral Arthritis). These ten common diseases are by no means the only diseases seen today in the turkey industry, either specifically in breeders or the commercial industry but, they are common and in some cases, less emphasized. That is in comparison to some reportable diseases such as Avian Influenza and Newcastle Disease, which have been reviewed and discussed ad nauseam.

Current Turkey Diseases in Australia

Dr Sheridan Alfirevich

Baiada Poultry

We are fortunate in Australia that we do not have many of the serious diseases that are endemic in turkeys internationally. Disease in turkeys in Australia occurs relatively infrequently but the clinical manifestation can be severe. Preventing and controlling disease often relies on understanding the inciting causes and management stressors, particularly as vaccination of turkey broilers for endemic diseases is not practically or commercially feasible.

Turkey diseases in Australia will be reviewed in the following broad categories:

- Respiratory diseases which may be caused by a primary pathogen or be mutli-factorial;
- Bacterial diseases such as: fowl cholera; erysipelas; and collibacillosis;
- Viral diseases such as: haemorrhagic enteritis virus (HEV); fowl pox; and avian influenza;
- Protozoal diseases such as histomoniasis; and
- Miscellaneous conditions such as: pendulous crop; leg disorders; poultry early flipover syndrome; and blepharoconjunctivitis.

Current Duck Diseases Internationally

Dr Alice Ghibaudo

Grimaud Freres

Duck breeding represents the 4th largest poultry production in the world. Different species are reared for meat production (pekin duck, muscovy duck, white goose) or for fat liver production (mule duck, grey goose).

This presentation is a non-exhaustive summary of the main diseases encountered on duck farms. The point of view adopted is that of field observation on broiler ducks, fat liver producing ducks or breeding ducks farms. The objective is to establish a reference base of the most frequent pathologies as an aid to the diagnosis and management of those diseases.

Current Duck Diseases in Australia

Yuanshuo Karen Gao

Zootechny Pty Ltd

Ducks are relatively disease resistant when comparing to chickens. Most of the infectious agents that would affect ducks in Australia are bacterial in origin, whereas viral duck diseases are rarely diagnosed in Australia. In addition, non-infectious diseases have also been seen in Australia, some relating to nutritional or management causes.

The most significant bacterial pathogens in Australia duck industry would be *Pasteurella multocida* and *Riemerella anatipestifer*, which can cause significant bird and production losses in unvaccinated flocks. Both diseases are responsive to antibiotic therapies and can be controlled by autogenous vaccines and improved hygiene. Colibacillosis are usually diagnosed as a secondary or concurrent disease, very occasionally as a primary pathogen. Ducks are also susceptible to *Salmonella* spp., similar to the broiler and layer industries. Clinical salmonellosis can occur if birds are infected young, otherwise salmonellosis is mainly a food safety disease, which can be managed with vaccination. A case of bacterial salpingitis involving multiple bacterial agents including *E. coli*, *Gallibacterium anatis* and/or *Trueperella pyogenes* was reported in 2019.

Viral diseases are rarely found in ducks in Australia. Avian Influenza had been previously diagnosed in commercial duck operations decades ago, there had been no recent reports of AI detection in the Australian duck industry. Currently Duck viral hepatitis and Duck viral enteritis are considered be exotic to Australia.

Leg weakness had been reported in broiler ducks, possibly similar aetiology to the skeletal and developmental diseases that seen in broiler chickens, factors including genetics, nutrition, growth rate and/or incubation profiles can be involved. Another developmental disease in broiler ducks is ascites, where genetics, growth rate and environmental conditions had been speculated to be involved.

Given the unique biosecurity status of Australia poultry industry, the duck industry in Australia is faced with much less disease challenges compared to overseas which is fortunate. The good hygiene and husbandry standards, strong biosecurity and welfare standards, improved genetics and nutritional profiles are the main focus of diseases and production management of the Australian duck industry.

ILT Control in SA

Margaret Sexton

Biosecurity SA, Dept of PIRSA

Infectious Laryngotracheitis (ILT) is an upper respiratory tract infection caused by a herpes virus, mainly affecting chickens. It is a notifiable disease in Australia, but endemic. The disease in unvaccinated or improperly vaccinated chickens can cause high mortalities and significant economic losses. Although there is a single serotype, various strains are identified by genomic characterisation and these have been classified into Classes 1 - 10. There are 3 vaccines available in Australia and all are live attenuated vaccines. Two are Class 1 strain (A20 and SA2) and the third is Class 7 strain (Serva). In birds that have been vaccinated or recovered from the disease, the virus remains latent but may be excreted at any time, usually when birds are stressed.

In SA there have been sporadic outbreaks of ILT in broilers up to 2004. These outbreaks were usually contained in one or two farms and eradicated within 1-2 months. The outbreaks often commenced in unvaccinated broiler farms located in close proximity to ineffectively vaccinated layer farms (particularly with SA2 vaccine). Infected broiler farms then spread virus to other farms en route to processing. Improved vaccination on layer farms, quarantine and rapid slaughter out of infected broiler farms, and cleaning and sanitation quickly resolved the issues.

From 2004 to 2018 the broiler industry in SA has had enormous growth – from 40 million to 138 million birds slaughtered per annum. This increase in bird numbers has also come with significant changes in farm size and configuration, including the construction of large complexes that can have up to 7 separate bio-secure units within the one complex (some of which are free range enterprises). One issue is that roadways on some of these complexes pass within 20 – 200m of other units.

In September 2018 an ILT outbreak commenced in commercial broilers in Murray Bridge. It was identified to be Class 7 strain. It was suspected that that source was from spent breeder hens which had been vaccinated with the Class 7 vaccine, and had passed close to the affected farm when first pickup was occurring. The outbreak then gradually spread to infect many other broiler farms in the area and also to a free range growing area over 125km away.

All broiler companies in SA examined their risk areas and put measures in place to try to minimise these risks. During the course of the disease outbreak the critical areas and ways of addressing them were identified. The layer industry was also impacted and they also addressed biosecurity issues.

PIRSA personnel assisted in disease investigations, notifying industry and all the ancillary businesses affected, and addressed issues arising with other government departments such as EPA, Road Transport and councils. They also assisted in routing

suspect infected stock trucks, manure trucks, and dead bird trucks away from other poultry farms. This required great cooperation and patience from trucking companies.

Other critical areas were the catching operations, farm operations, live bird receival at the processing plant, and also the planning around these activities. All operational areas were audited regularly to ensure that changes made were working and agreed standards adhered to.

In the catching operations the areas addressed were the catchers' hygiene between each farm caught in a night – requiring clean clothes, boots gloves etc between farms. Extra forklifts were hired and placed on each new farm to be caught. The forklifts remained on the farm until finished and were then taken away for cleaning and sanitisation (in daylight hours!). It was found that forklifts are not designed to be easily cleaned and hiring extra forklifts was actually cost-effective. Modules and trailers were also separated into dedicated small and big bird equipment.

At the processing plant, the cleaning and sanitising of crates, modules, trailers and trucks was upgraded. Separation was emphasised between trucks and modules carrying incoming live (infected) birds and cleaned and sanitised equipment and trucks. Particular attention was paid to forklifts again to ensure separation of dirty from clean, as well as effective cleaning.

On farms, cleanouts, dead bird disposal, and litter removal were targeted. The movement of personnel from farm to farm both from the company and also the owners and workers was also limited. This was particularly an issue on large complexes with birds of different ages. Ensuring adequate turn-around times for cleaning were also targeted so that all sheds were cleaned before sanitation started. Vaccination using Class 1 vaccines was used initially on all farms but then reduced to only farms getting re-infected. Correct vaccination procedures were absolutely critical.

Planning involved much of the above issues and also ensuring that non-infected farms were caught before suspect and vaccinated stock. Thinning out was reduced and part sheds were completed by the end of the week. Infected farms were finished at the ends of shifts so effective cleaning of equipment and personnel could occur.

The last notified case was in December 2019 (with a total of 83 notifications). Vaccination continued on several complexes until dust samples collected during the batches indicated that no Class 7 could be detected – only Class 1. Vaccination ceased entirely from July 2020. Further testing of dust samples also indicated that Class 1 virus was absent in subsequent batches.

This outbreak and its resolution shows that it is possible to get rid of ILT from broiler operations, but it requires careful thought, investigation of solutions in the areas of cross-contamination and decontamination. It requires collaboration of all parties involved through chain, and support where required by government agencies and councils.

Practical lab techniques to assist field control of ILT

Stephen Walkden-Brown[1], Ashley Etherington[2], Awol Assen[1], Sarah Williamson[3], Priscilla Gerber[1], Sue Sharpe[3] and Peter Groves[4]

[1]School of Environmental and Rural Science, University of New England, Armidale, NSW 2351.
[2]Ingham's Group Limited, Salisbury South, SA 5106.
[3]Birling Avian Laboratories, Bringelly, NSW 2556.
[4]School of Veterinary Science, Poultry Research Foundation, The University of Sydney Camden, NSW 2570.

The Problem

Mass vaccination of broilers against ILT in water is a complex multifaceted process that is prone to failure (Groves et al. 2019). Poor initial vaccine take is accompanied by bird to bird transmission of the vaccine virus with the attendant risks of delaying full flock protection, reversion to virulence (Guy et al. 1991) and recombination between competing ILT viruses to create more virulent strains (Lee et al. 2015). Monitoring the success of vaccination has been hampered by reliance on traditional diagnostic methods (eg. antibody response) based on individual bird sampling. The costs of effectively monitoring vaccination outcomes this way mean that it generally does not occur. Our goal was to determine if a relatively inexpensive population level sampling strategy could assist with assessing vaccination outcomes and strategies for ILT control. We chose to focus on qPCR analysis of poultry dust samples, based on previous success with this approach in evaluating Marek's disease status (Walkden-Brown et al. 2013). A team of researchers at UNE, USyd, Birling Avian laboratories and collaborators in industry, with funding from AgriFutures Chick Meat program (see acknowledgments) has worked systematically towards evaluating this approach and we now have a clear take on its utility. Our talk will overview the research and provide a case study of application in the field.

How we researched the problem and results found

Field validation of dust testing. We knew from earlier work that ILTV is shed copiously in feather dander and excreta following vaccination (Roy et al. 2015). We then set about evaluating whether this could be useful in the field, working with 8 flocks of broilers vaccinated with the Serva vaccine and monitoring ILTV in both upper respiratory tract swabs and dust. This work revealed that the level of vaccine take at 4 dpv assessed by swabs was pretty low, <55% in all flocks and <20% in half the flocks (Groves et al. 2019). The subsequent profile of positive birds out to 25-26 dpv led the vaccine take to be assessed as "poor" in 3 flocks and "better" in 5 of the flocks. Evaluation of settle plate dust samples collected during this work showed that the poor takes could be readily predicted by low ILTV genome copies (GC) is dust samples collected at 7 or 8 DPV (Ahaduzzaman et al. 2020). This study also described a dust collection method based on settle plates attached to down wires in the shed and

showed that the amount of dust collected but not the ILTV GC content of the dust varied with location of the settle plates. It was recommended that 2 dust samples be collected from a shed at 7-8 dpv and then pooled to send of the laboratory for qPCR testing, resulting in one test per shed. A further study with A20 vaccine showed a similar association between dust ILTV GC at 7 dpv and vaccine take in tracheal swabs, indicating that the dust test was useful for both of the vaccine strains used in broilers in Australia (Assen et al. 2020).

Overcoming some hiccups with the dust testing. In the above studies, no wild type virus incursion was detected and the 7 dpv dust test appeared to have good utility for indirectly evaluating vaccine take. However as a wider range of farms was dust sampled it became apparent that some farms showed high levels of ILTV GC in dust collected on the day of vaccination (prior to actual vaccination). Typing of these samples in some A20 (ILT Class 1) vaccinated flocks revealed the presence of class 7 and/or Class 1 virus, suggestive of circulating virus other than the vaccine virus, or contamination by the vaccine virus (Assen et al., unpublished data). These findings were surprising because they suggested quite widespread circulation of ILT virus in flocks by the time of vaccination at 7-14 days of age, without necessarily resulting in clinical signs or outbreaks. It was also clear that should this be the situation on a farm, a 7 dpv dust test is not informative about the success of vaccination. The recommendation for vaccination assessment was therefore modified to have settle plates installed at placement, with dust samples collected on the day of vaccination (0 dpv) and at 7-8 dpv. Thus analysis of two pooled samples rather than one, is required to have a meaningful assessment of vaccine efficacy. A significant benefit of this approach was found to be the alerting of industry to problems with circulating ILT in their flocks prior to vaccination.

A case study. Ingham's Group in South Australia had collaborated closely with the project and had run into the problem of circulating class 7 virus in areas with only A20 (Class 1) vaccination during the dust testing. At the time ILT was spreading rapidly and easily and there was uncertainty as to whether the test was detecting viable virus (current or carryover infection) or non-viable residues from prior batches, post sanitation. Differentiation of field infection from vaccination reactions also had to be conducted and this was achieved by collecting swabs as well as dust samples on the day of vaccination and by typing of ILTV in swab and dust samples. This confirmed the presence of circulating class 7 early in some chicken batches.

A comprehensive ILT control program, incorporating an A20 vaccination program and quarantine and biosecurity measures was implemented (Margaret Sexton, these proceedings). The modified dust sampling protocol was used to verify that vaccination was effective and vaccination techniques were refined. The use of dust sampling at day of vaccination, from collection devices installed at placement, then began to confirm the absence of ILT virus prior to vaccination.

As clinical cases subsided, vaccination was reduced – initially all farms were vaccinated, but later vaccination was restricted to aggregations of farms in relatively close proximity. This became possible when the enhanced biosecurity procedures proved capable of eliminating physical spread from one complex to another. All vaccination was finally stopped and a comprehensive analysis of dust samples from 50 sheds across the growout was conducted, seeking evidence of the presence of

subclinical infection. The fact that all samples tested negative provided sufficient confidence for Ingham's group to unwind some of the more costly biosecurity measures.

Other relevant research findings and points

1. **Does the ILTV GC detected by qPCR represent infectious virus?** Our findings from several studies indicate that the vast majority of the ILTV GC detected in dust represents non-infectious or inactivated virus. We have been unable to isolate ILTV from dust samples from infected chickens in cell culture or chick embryos (Bindari *et al.* 2020). We have also been unable to infect chickens with eye drop administration of dust extracts or insufflation of dust into the upper respiratory tract of susceptible chickens (Yegoraw *et al.* 2021). We have shown that the major component of poultry dust is aerosolised excreta (Ahaduzzaman *et al.* 2021) and extracts of excreta from infective birds do not transmit ILTV (Yegoraw *et al.* 2021) strongly suggesting that the GC detected in excreta represents inactivated virus and that this constitutes a major proportion of the ILTV GC detected in dust samples.

2. **How stable is the ILTV DNA in dust samples and what are the requirements for storage and transportation?** The DNA from ILTV (and RNA from IBV) are very stable in dry dust samples and can be held and shipped at room temperature with no loss of detection (Tran *et al.* 2021). These authors showed that dry dust can be stored at any temperature up to 37˚C for at least 4 months without loss in qPCR detection of ILTV or IBV GC. Collection or storage of moist dust should be avoided or air drying prior to storage is recommended if only moist dust is available.

3. **Can ILTV virus be typed from dust samples?** The qPCR tests used to detect ILTV in dust samples do not differentiate between vaccine and wild-type virus. To identify the class of virus involved, an additional step of typing the virus into different classes is required (Kirkpatrick *et al.* 2006). This can be done using DNA extracted from dust although it is more difficult than from swab or tissue samples. We have found that a minimum ILTV load in dust of 10^5 GC/mg dust is required before DNA extraction and typing from a dust sample should be considered.

4. **What is the best and easiest way to collect dust samples?** Dust samples scraped from surfaces in the shed can provide useful information, but are not recommended as the surface may contain residual viral DNA from previous batches or of a historical nature. We recommend the collection of settled dust on specially designed collectors which attach easily to down wires in the shed. Our original settle plate design had drawbacks in the plates being dislodged by wind, people or equipment at times and the need to transfer dust from the settle plate to a ziplock bag (often with loss of sample, and potential contamination). This has been superseded by a new funnel shaped dust collector that directs dust into a dust collection vial screwed on at the bottom of the collector. By tapping on the side of the funnel, settled dust is displaced into the collection vial which can simply be removed and a new cap placed on it ready for shipment to the lab. We have shown in several studies that the location of the dust collector in a shed does not affect the pathogen load in dust samples (Nguyen *et al.* 2019; Ahaduzzaman *et al.*

2020), but more dust is collected towards the extraction fan end of tunnel ventilated sheds, so the recommendation is to place two collectors towards that end of the shed, and send a pooled sample from these to the lab. Dust deposition rates in sheds are around 10 mg/100cm^2/day being lower during the very early post placement period and in tunnel ventilated compared to conventional sheds (Ahaduzzaman *et al.*, 2020). The qPCR test is based upon 5mg of dust.

5. **Where can the dust samples be tested for ILTV?** The poultry health lab at the University of New England offers a commercial service for qPCR analysis. Guidelines, pricing and submission forms are available from the first author on this paper. Birling Avian Laboratories also has the equipment and PCR tests available to conduct this testing.

Implications/conclusions

- The dust test for ILTV offers a simple method of sample collection that can be done with unskilled staff
- Sample prep and transfer to the lab is easy, simple and cheap. Turnaround times for results are quick
- Because of its relatively low cost and stability of the samples, it can be used to verify vaccination efficacy and for epidemiological investigations where one would usually need a large number of samples
- For the integrator it can be used as a “proof of freedom” tool. Vaccine availability has been constrained and one is reluctant to reduce/stop future orders without confidence in the field situation. Sometimes symptoms may be very mild and one can miss positive cases as in outbreak scenarios, personnel movement is restricted and one relies on farm managers to report symptoms.

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Interpretation of Field Epidemiological Studies – advantages and hazards.

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The Problem

Structured field epidemiological studies are underutilized in veterinary medicine. The intention of this presentation is to briefly outline the usefulness of these types of studies and to point out some hazards in their use (or abuse). It is presented as a lead I to Yuanshuo Gao's presentation (these proceedings) on preliminary findings within the Spotty Liver Analytical Epidemiology project currently funded by Australian Eggs.

Many (probably most) diseases are multifactorial. Well-designed epidemiological studies can help unravel these complex issues. These studies search for risk factors (that are associated either with the disease occurrence or with its absence), but the true underlying factors that contribute to disease presentation are often hidden, confounded or misconstrued. While causation of disease is almost impossible to determine from an epidemiological study, the information gained will often lead to a more efficient and focussed experimental follow up to improve understanding. With this in mind, epidemiological studies offer much and should be used early in the outbreak of something new or emerging.

Epidemiology?

There are two basic types of epidemiological study. The first is "Descriptive" which gathers information on the "what, who, when and where" of a disease. Useful early on in a new situation, this is what most people think of when the word "epidemiology" is used. This aids in defining the disease's life history, which hosts are affected (breed, age, sex etc) and where and when it occurs. A study should reveal figures on disease rates (e.g. incidence, prevalence, incidence density, attack rates, etc). Some good information in this sphere has been produced (Courtice *et al*., 2018; Gao *et al*., 2020). Analytical epidemiology however seeks to identify risk factors for a disease. Understanding these gives insight into why the disease occurs and why and how it may vary in its manifestations. These studies search for factors more often associated with the disease occurrence or with its non-occurrence (i.e. protective factors). Determining these can often lead industry to ameliorate or even avoid the disease altogether.

Identifying valid risk factors can be difficult as there are often many confounding or interacting factors involved. These require considerable care and good statistical analysis to sort out. The overall goal being to identify one or more "Key Determinant" of the disease. A key determinant is a factor which affects the outcome of a disease

and can be controlled by management. Finding and understanding some of these can greatly improve disease control and animal welfare in the field.

Confounding or Coincidence

It is easy to jump to a conclusion in the field if the real likelihood of coincidence is underestimated. Coincidence is extremely common and often misunderstood and falsely associated with other outcomes. A few amusing examples will be shared in the presentation. Wrong assumptions can lead poor management and wasted effort in disease control measures. This is one reason why the "Control" units in a study are so important. When we investigate disease, we tend to focus on the animals or farms which have the disease. It is probably even more important to look at why some animals or farms DO NOT have the problem. Confounding is a particular problem. This occurs when there appears to be an association of a factor and the disease, but the truth is that there is another factor, either known or unknown, which is associated with both the incriminated factor and the disease. Finding these can take considerable analyses and skill.

Study design and sample size

Sample size in a study needs to be large, but this is limited by your resources. Funding and people time (and travel restrictions in 2020!) will limit the size of the study possible. If you're using expensive tests as part of the study, your sample frame becomes more limited. Making as efficient a study that gives valuable results is difficult.

Implications/conclusions

Well-designed epidemiological studies can provide valuable and practical outcomes for disease control, especially when new situations or re-emergence occurs. Epidemiology should be employed early in a disease investigation as they can more efficiently guide further experimental studies to better understand a condition.

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Spotty Liver Disease and Risk Factors

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Over the last 18 months, an observational epidemiology study was conducted in free-range laying flocks across different states of Australia, in an initial attempt to identify risk factors for the occurrence of Spotty Liver Disease (SLD). Due to the impact of COVID-19 on domestic travels, only 24 laying flocks were sampled in the end, which unfortunately was greatly reduced from the initial sample size of 32 flocks.

Approximately 230 variables were analysed in a case-control analysis, covering data collected in both rearing and laying, including bird history, farm and shed design, bird health, nutrition, biosecurity measures etc. A 'case' was defined as any farm with clinical SLD with characteristic postmortem signs with an increase in mortalities or a drop in production. A 'control' was defined as any farm that did not have clinical SLD.

	No scratch area	Scratch area
Case	**5**	**13**
Control	**6**	**0**

A putative sufficient cause of SLD was identified, which requires the presence of C. hepaticus and scratch areas in the laying shed (n=24, P=0.003). This is significant finding which also makes biological sense. Effectively, in laying sheds that had no scratch areas, i.e. fully slatted sheds, birds were are less likely to be in contact with faecal matters contaminated with *C. hepaticus*. It is speculated that the faecal matters in the range are less concentrated in *C. hepaticus* and that *C. hepaticus* infectivity might change once exposed to unfavourable conditions i.e. oxygen.

In order to further investigate the risk factors for SLD in fully slatted sheds, we narrowed down our data which further limited our sample size (n=11). However, we found the following potential risk/protective factors:

1) Smaller flock size is more at risk of SLD (P=0.002)?
2) Less useable floor space (P=0.08) – confounded with flock size?
3) Cool white lights in lay are protective (P=0.143)?
4) Slat design that has bigger holes are protective (P=0.18)?
5) One particular breed is more at risk (P=0.061) – small sample size?

This initial phase of the study was designed to capture as much information possible, which resulted in very extensive questionnaires, however it provided us with some valuable information for the design of next phase which to be carried out in the coming months.

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The impact of toxigenic moulds and mycotoxins in poultry feed

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Mycotoxins have emerged to the most prominent undesirable feed contaminants worldwide. The increase in the prevalence seems to be related to changes in agricultural practice, which modifies the soil microbiome and increases the susceptibility of plants to fungal invasion. In addition, the global climate change, with extreme temperature and rain fall certainly contributes to plant stress. Although plant diseases caused by toxigenic moulds are recognized for many years, only in the last decade, studies of plant-fungal and mycotoxin-plant interaction have been studied particularly for Fusarium species. These studies revealed that plant tissues modify mycotoxins mainly by conjugation reactions with glucose or amino acids. These conjugations reaction modify the chemical physical properties of a mycotoxin, and hence the conjugated forms are not identified by common analytical procedures. In the early the term *masked mycotoxins* was coined to explain the obvious differences between experimental studies with purified mycotoxins, and the field observations with natural contaminated feed material. The insight in plant-mycotoxin interactions now explain that the conjugated and modified form occurring in naturally fungal-infected plant material contribute to animal exposure, as the conjugates are cleaved by the intestinal microorganisms, resulting in the realise of the biologically active mycotoxin.

In daily practice, animals are exposed in most cases to mixtures of mycotoxins, depending on the ingredients included in their diet. Professional feed processor are aware of the adverse effects of mycotoxins and follow the recommendation that limit the concentration of individual mycotoxins to levels that are considered as safe for the animal. The statutory maximum levels are based on controlled experimental studies with individual mycotoxins and aim to prevent any clinically adverse effects. This pragmatic approach can not sufficiently predict the exposure scenarios and consequent adverse effects occurring in daily practice. It is well-documented that synergistic effects occur between different mycotoxins and hence poultry producer and veterinarian have to face the challenge of undesirable effects on poultry health associated with the exposure to low concentrations of multiple mycotoxins.

Mycotoxins are chemically very diverse and hence their toxicological effects are diverse as well. From the beginning of mycotoxin research starting with detection and characterizations of the aflatoxins, the liver has been identified as one of the major target organs. Organ-specific pathologies include bile-duct proliferation and hepatocellular necroses, caused particular by the Aflatoxin-epoxide that is generated by biotransformation enzymes (CYP450 family) in the liver of the animal. As the expression of these CYP450 enzymes varies between species, the actual formation of the aflatoxin epoxide varies also explaining not only the difference in species

vulnerability (even within avian species), but also the individual susceptibility, as a plant-based diet may contain plant metabolites that modify the expression of the biotransformation enzymes. An impairment of liver cell functions results on a decrease of metabolic and synthetic functions. These include among others in a decrease in the synthesis of functional proteins, resulting for example in an impaired blood clotting and haemorrhages. Similar detailed description of the mechanism pf action can be given for nephrotoxin mycotoxins, such as ochratoxin A. The latter accumulates specifically in proximal tubule cells causing a functional impairment associated with high renal fluid and protein losses. Less prominent endpoints of toxicity are the muscle and bone tissue, which in turn can affect carcass quality. Individual mycotoxins have been also tested for their adverse effect on male and female fertility, embryotoxicity and specific other target tissues, such as the well-known dermatototoxicity (beak lesions) and haematotoxicity (pancytopenia) of T-2 toxin.

The detrimental effect of mycotoxins on the immune system is considered as one of the hidden but important effects in daily practice. Again, the early literature described in detail the alterations in the spleen and bursal tissue following mycotoxin exposure. These obvious histopathological changes could be experimentally induced by relatively high dosages of individual mycotoxins. Recent interest focusses on the adverse effects of mycotoxins on the intestines. The intestines are not only the first contact site for mycotoxins with a poor bioavailability in poultry (such as for example deoxynivalenol) but also harbour 70-80 % of the active immune cells. Different investigations could now show that all major mycotoxins (aflatoxins, ochratoxins, trichothecenes (Fusarium toxins) affect the integrity of the intestinal barrier causing a leaky gut syndrome. Such an impairment of the intestinal barrier facilitates the transfer of antigens, microbial toxins and pathogens into the intestinal tissue and beyond, resulting in an overall inflammatory response. Oxidative stress and inflammatory mediators affect the humoral and cellular immunological defence mechanisms. The overall outcome is an increased sensitivity to viral, bacterial, and protozoal diseases, and a risk of vaccination failure due to an impaired immune response.

The short summary of some of the recent considerations and insight in the diverse effects related to the contamination of animal feeds with mycotoxins should underlines the need to include the risk mycotoxin exposure in daily poultry practice and poultry nutrition and health management.

Salmonella – longitudinal survey and use of probiotics

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Non-typhoidal *Salmonella enterica* serovars are among the most common causes of foodborne gastrointestinal disease. Raw or undercooked eggs or egg-containing food items are frequently implicated as sources of *Salmonella* during the investigation of outbreaks and sporadic cases of gastrointestinal disease. A longitudinal study on single-aged caged layer hen flocks for their lifetime showed that chicks were *Salmonella* negative at hatch and remained negative during rearing. Prior to placement of pullets on the production farm, residual *Salmonella* was detected in dust samples. Pullets turned positive after their introduction to production farms. Throughout the study, environmental samples (dust and egg belt) were most likely to be *Salmonella* positive. The longitudinal investigation on free-range farms showed that the levels of *Salmonella* were low. Detection of the bacteria was intermittent. Dust and nest box swabs were the most common sampling sites to be positive for *Salmonella*. *Salmonella* was detected on egg grading equipment after processing both free-range and caged eggs. Bacteria were only detected on suction cups used to move eggs from trays to the conveyor belt prior to washing. There are multiple farm intervention strategies to reduce the risk of Salmonella. Strategic feeding of probiotics and synbiotics is one of the intervention strategies for enhancing food safety. The short-term trials in chicks showed that short-term feeding of both the probiotics and synbiotics were effective in improving the gut microbial balance displaced by the *Salmonella* Typhimurium challenge, but the products were not effective in significantly reducing *Salmonella* shedding level or invasion into internal organs. The long-term pen trial with the *Bacillus* based probiotic revealed that continuous feeding of the probiotic was effective in reducing the faecal and organ load of *Salmonella* Typhimurium and balancing the microbial communities displaced by the challenge. Periodic feeding (four weeks on and four weeks off) did not result in the reduction of *Salmonella* Typhimurium shedding which suggests that continuous feeding is beneficial. Partly, probiotics and synbiotics supplementation can be an effective strategy for improving gut microbiota that in turn enhances food safety. Further studies are essential to understand the effects of probiotics on the gut microbiota of hens raised in different housing conditions.

Seasonal variation of key food safety pathogens in the processing plant

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Introduction

Food safety is a key issue for all food industries, including chicken meat. *Campylobacter* is one of the food safety pathogens that is a major cause of human gastroenteritis which is mainly, but not necessarily exclusively, linked to chicken meat. The reality is that the significant food safety pathogens (*Campylobacter* and *Salmonella*) are normal flora that can be present in the gut of healthy meat chickens. Recognising this reality, the chicken meat industry has had a long history of active research into improved methods to reduce and eliminate these pathogens on-farm and in the processing plant.

This project seeks to provide key information in two areas. Firstly, a national snapshot of the prevalence and levels of two key pathogens (*Campylobacter* and *Salmonella*) in representative processing plants Australia-wide. Secondly, the natural variation that occurs in the presence and level of the same pathogens over time (at the seasonal level and at the day level).

Research Methods

The basic methodology for the project was:

- six representative processing plants were selected based on interventions currently in place to reduce key food safety pathogens (*Campylobacter* and *Salmonella*), with the advice of the industry steering committee
- intensive sampling twice in the year for each plant, in winter (May-August) and summer (October-February)
- repeat sampling on three consecutive days in a week for three consecutive weeks in a row at each plant in winter and summer
- each day 10 chickens were sampled at two key points in the processing plant, individual caeca (at the point of evisceration) and carcass rinses (after chill/before packaging)
- the presence and levels of *Campylobacter* and *Salmonella* were determined using Australian Standard Methods where available.

Results

Of the samples collected during this study, 93.5% of caeca and 69% of carcass rinse samples were *Campylobacter*-positive. The mean *Campylobacter* count in the caeca was 6.87 $\log_{10}$ cfu/g in winter and 7.28 $\log_{10}$ cfu/g in summer; and in carcass rinses was 3.97 $\log_{10}$ cfu/ml in winter and 3.93 $\log_{10}$ cfu/ml in summer.

For *Salmonella*, 2.7% of caeca and 5.4% of carcass rinse samples were positive. The levels of *Salmonella* in individual samples for this study were quite variable. In the caeca, the levels were between <3 to 1100 MPN/g whilst in the carcass rinse samples the levels were between <0.3 to 110 MPN/ml, with 56.5% of samples recording <0.3 MPN/ml.

For *Campylobacter*, statistical analysis shows that summer had a significantly greater caecal *Campylobacter* count than winter, with processing plant and season both significant but not highly so. In particular, one processing plant had a significantly higher caecal *Campylobacter* count than other processing plants while one processing plant was significantly lower than other processing plants.

When carcass rinse samples were analysed there was no significant difference between the variables of day or season, unlike what was observed in the caeca for season. However the processing plant was significant with one processing plant having a significantly higher count in the carcass rinses. These results indicate that there is seasonal variation in *Campylobacter* counts of flocks entering the processing plant but once the chickens have been processed, seasonal difference disappears.

A very low rate of *Salmonella* was detected in the samples in this study, so the results should be assessed with care. Statistical analysis shows there was no significant differences for processing plant or season in the caeca but in the carcass rinse samples processing plant was found to be significantly different.

In summary, this study demonstrates there is a seasonal variation for *Campylobacter* in the caeca. The data shows that summer had a significantly greater caecal *Campylobacter* count than winter. However this same seasonal variation is not observed in the carcass rinse samples at the end of processing. Processing plant as a variable was consistent across both caeca and carcass rinses with one processing plant having significantly higher levels of *Campylobacter*-positive samples than other processing plants. There was no seasonal variation observed for *Salmonella* in either the caeca or the carcass rinse samples although once again processing plant was significant for the carcass rinses. However, it must be noted that only one winter and one summer was studied for each processing plant. Our observations may simply be what occurred in 2019/2020 seasons.

Implications

This study has several important implications:
seasonal variation occurs at the flock level, with chickens entering the processing plants having significantly higher caecal *Campylobacter* counts in summer than winter seasonal variation did not occur in carcass rinse samples of chickens collected post chill

processing plant variation is significant for both the caeca and the carcass rinse samples for *Campylobacter*; and the carcass rinse samples for *Salmonella.*

Recommendations

This report provides up-to-date knowledge of the presence and current levels, as well as seasonal and daily variations of *Campylobacter* and *Salmonella* in representative processing plants from major companies within Australia
Further studies need to include more than one yearly cycle of seasons to elucidate consistent seasonal trends for different processing plants.

Fowl cholera – genomic analysis of *Pasteurella multocida* isolates to investigate outbreak dynamics

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Phase variation is a reversible mechanism of switching of gene expression. It is mostly associated with those genes coding surface structures of bacteria such as the lipo-oligosaccharide of *Haemophilus influenzae (1).* There has been no published record of phase variation mechanism in switching of the glycosyltransferase gene expression of lipopolysaccharide (LPS) outer core biosynthesis loci in *Pasteurella multocida* [9]. LPS is one of the most important immunogenic virulence factors of *P. multocida*. Recent works have demonstrated that killed vaccines give protection only against strains with identical or nearly identical LPS structures. Eight LPS genotypes (LPS1 to LPS8) have been recognised in *P. multocida*, based on their LPS outer core biosynthesis locus (2). LPS3 has been recognised as the most prevalent LPS type associated with fowl cholera in Australia (3).

In this study, we used whole genome sequencing (WGS) and phylogenomic analysis to investigate the relatedness of sequence type (ST) 20 isolates across the years within two free range layer farms. As well, the genomic data was used for monitoring and comparing the variations in the lipopolysaccharide (LPS) outer core biosynthesis loci of ST9/LPS3.

A total of 73 isolates from two different free range layer farms were included. Our genomic analysis revealed that all investigated isolates within the two farms (Layer A and B) carried LPS3, albeit with a high degree of genetic diversity between them. Additionally, the isolates belonged to five different sequence types (ST) with isolates belonging to ST9 and ST20 being the most prevalent.

Phylogenomic analysis of the ST20 isolates obtained from 2 separate outbreaks (1994 and 2002) in Farm A identified two separate populations. Comparison of the ST20 isolates from Farm A to those of a distantly located farm, identified that the 1994 Farm A isolates were more closely related to isolates from Farm B, than to the 2002 Farm A isolates. Together these results suggest a common source for outbreaks on Farm A in 1994 and Farm B.

Some isolates carried ST-specific mutations within their LPS3 genetic region, including frameshift mutations in the galactosyltransferase gene in those of the ST20s. The

LPS3 genetic region carried by ST9 isolates could be separated into 3 subtypes with evidence for 2 potential phase variation mechanisms identified. The first ST9 variant is defined by a 7 bp sequence insertion (TTA TTA T) in *natC* (position 730). This mutation is single copy duplication of one half of an imperfect tandem repeat, producing a three-unit tandem repeat array (5'-TTAATAT-TTATTAT-TTATTAT-3'). The second variant is a one bp deletion at position 722 of *gatG* (721_723delA) in a homopolymer track. Importantly, our results demonstrated that these two LPS3 variant shared identical rep-PCR patterns, making predicting the protectotype based on the currently used typing techniques impossible. In addition, we found the presence of a mixed population of ST9/LPS3 *P. multocida* during a single outbreak with different switching of their *natC* gene resulting in different LPS outer structures. Our results strongly suggest the need for a genetic typing scheme for LPS to ensure an appropriate vaccine strain with a matching predicted LPS structure is used. This would need culture independent typing of *P. multocida* population within a flock to be able to pick this population level diversity.

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Video analysis of flock motion in commercial sheds for health prediction

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Early prediction of flock health provides opportunity for interventions to enhance welfare and production. Camera technologies with real-time automated image analysis have potential to complement human inspections and generate flock health alerts. Research literature has demonstrated the use of cameras to provide precision and automatic detection of: space utilisation in sheds; health indicators of footpad dermatitis, hockburn and bacterial infections; and chicken body size for weight estimation. To date, camera systems have provided measurements in units that are not easily transferable to different sheds or flocks, or require lengthy calibration.

This project has evaluated image analysis approaches of the two major existing commercial camera systems, and developed refinements for Australian shed conditions that address variations in lighting, chickens sitting close together and measurement of physical sizes. The developed automated image analysis enables camera-based flock health indicators and chicken body size to be expressed in physical units. Trials are continuing this year for validation on additional flocks.

Welfare Aspects of HatchCare

Ashley Etherington

Veterinarian, Inghams Enterprises

HatchTech markets the HatchCare hatcher which provides the hatched chick with light, feed and water in the hatcher machine. Inghams is currently constructing two HatchCare hatcheries – in Melbourne and Perth.

The chicks within the HatchCare hatcher have more space and a better environment than that provided by a traditional machine.

The hatched chicks stay in the hatcher basket all the way to the farm, eliminating the traditional mechanical handling processes like the chick separator, shell separator, counting machine and associated transfer conveyor belts. This is considered to be a significant change to the welfare of the newly hatched chick.

The benefits of early feeding on gut health and immune function are well known and are believed to contribute to what I call the “commercial benefits”.

The purported commercial benefits of the system are:

- Increased day old weights
- Improved mortality
- Faster growth
- Improved FCR

It is suggested that although these commercial drivers may underpin the basis of an investment in HatchCare, there are significant welfare benefits which could contribute to the decision.