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Assessment of the potential relationship between egg quality and infectious bronchitis virus infection in Australian layer flocks

KA Hewson,[a]* T Robertson,[a] PA Steer,[a] JM Devlin,[b] AH Noormohammadi[a] and J Ignjatovic[a]

Objective This investigation aimed to determine if there was a relationship between the production of eggs with poor internal quality, as measured by poor Haugh units, by Australian layer flocks and the detection of infectious bronchitis virus (IBV) in the hens. Other risk factors including flock size, flock type, flock age, chicken breed and vaccination frequency were also assessed.

Methods The study group comprised 17 flocks from 14 farms. Data relating to the factors investigated were requested on a regular basis. The Haugh unit data were used to grade eggs as good or poor based on the age and flock at the time of data collection. Cloacal swabs were collected from 20 chickens in each flock approximately every 6 weeks.

Results IBV was detected from a majority of the flocks and in 68% of cases the IBV strain detected was an A-vaccine-related field strain. Three variant strains were detected. Detection of IBV in a flock, the farm type and flock size were identified as potential risk factors for the production of eggs with poor Haugh units.

Conclusion IBV is prevalent in Australian layer flocks, but infection was primarily subclinical. The results complement previous reports indicating that there are many potential risk factors for the production of eggs with poor Haugh units.

Keywords infectious bronchitis virus; Haugh units; layer hens; risk factors; vaccines

Abbreviations bp, base pairs; HRM, high-resolution melt; IB, infectious bronchitis; IBV, IB virus; UTR, untranslated region

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Infectious bronchitis virus (IBV) is a group 3 coronavirus that is highly contagious and prevalent in all types of poultry flocks worldwide. IBV is the causal agent of infectious bronchitis (IB), which is characterised by respiratory, renal and/or reproductive disease.[1] Clinical signs may be associated with deaths of broiler chickens and reduced eggshell and albumen quality in layer hens, leading to economic losses for poultry farmers. Several different IBV strains have been isolated and classified[2–8] since IB was first identified in the United States in the 1930s.[9] Notably, IBV strains have been constantly diverging, although distinct and diverse sublineages can be exclusive to a single country such as Australia.[8,10,11]

*Corresponding author.
[a]Department of Veterinary Science, The University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia; khewson@unimelb.edu.au
[b]Department of Veterinary Science, The University of Melbourne, Parkville, Victoria, Australia

There is no treatment for IB, so prevention is the most effective way to control spread of the disease. As IBV transmission is airborne,[12] biosecurity alone is not always an effective control measure, so vaccination protocols involving the use of live virus vaccines are widely used.[13–17] Four live vaccines are available in Australia: vaccines I, S and VicS belong to antigenic subtype B[11,18] and vaccine A is classified as subtype C. The first Australian vaccine produced and applied was the VicS, which was developed from an Australian nephropathogenic IBV strain that causes nephrosis/nephritis in young chicks.[8] The original isolate from which the two other subtype B vaccines, S and I, were derived is unreported; however, nucleotide sequence analysis of their S1 genes indicates that all three vaccines are genetically identical.[19,20] The VicS vaccine has been used nationally since the 1960s to vaccinate 1-day-old chicks, primarily in broiler chickens and breeder flocks. Because inactivated IB vaccines have not been available in Australia, layer chickens were not vaccinated against IBV until recently because of concerns that live vaccines may affect laying performance and egg quality.

In Australia, there has been ongoing evaluation of the possible role of IBV in egg quality and production.[21,22] Circumstantial evidence has lead to the large-scale adoption of IBV vaccination in Australian layer flocks (Peter Scott, Scolexia Avian and Animal Health Consultancy, pers. comm.). Currently, it is estimated that most layer chickens are vaccinated with live IBV vaccines administered in drinking water at 8 and 16 weeks of age and often during lay. Meanwhile, the true relationship between IBV and any observed drop in internal and external egg quality and egg production remains largely unknown.

Internal egg quality is commonly measured using Haugh units as part of the routine on-farm quality control. High-quality eggs have high Haugh units, with thicker albumen, or egg white, in relation to total egg weight than low-quality eggs.[21] Factors reported to influence Haugh units include chicken breed, age, feed and disease status, in particular infection by IBV, the storage time and temperature of the eggs, and the presence of fungal toxins.[23–25]

Very early international observations showed an association between IBV infection and a decline in egg quality and egg production,[26] but the tropism of Australian strains of IBV for the reproductive tract, and the associated effect on egg quality and production, is unclear. Studies have shown that IBV may be associated with a decline in egg production and deterioration in shell and albumen quality, but with only mild or no respiratory signs,[27] and two Australian field IBV strains have been shown to reduce the quality and quantity of eggs produced by some hens.[28,29] Current Australian IBV vaccines have been shown to have no effect on egg quality or production.[30]

New strains of IBV arise commonly because of the frequent recombination of and spontaneous mutations in its single-stranded RNA.[3,31–33] Most Australian IBV strains characterised to date have been isolated from broiler chickens, with over 50 IBV strains belonging to a dozen serotypes identified within the past 30 years.[8,10,11,34–36]

At present, the most common method used for IBV detection and characterisation in Australian poultry involves PCR of the IBV 3′ untranslated region (UTR) followed by high-resolution melt (HRM) curve analysis.[19] Using this technique, three predominant subgroups of IBV have been established. Subgroup 1 strains include the vaccine and vaccine-related strains (>95% sequence similarity), subgroup 2 strains include the variant field isolates detected in the 1980s[10] and subgroup 3 strains include variant field isolates detected more recently.[7] All other strains are considered 'variant field strains'; however, vaccine A and V3/02 (which is the representative vaccine A-related field isolate, GenBank accession number DQ490217) -related field strains (>95% sequence similarity) could be considered a separate subgroup. Isolation of V3/02-related field strains from broiler chicken flocks has been common in the past 10 years, often associated with nephritis and increased mortality.[20]

Accurate detection and determination of the IBV types in Australian layer flocks is necessary for implementation of effective control measures and for understanding the epidemiology and evolution of IBVs.

This investigation used the PCR/HRM technique to detect and characterise IBV strains circulating in Australian layer hens in order to determine if the presence of IBV in a layer flock was related to reduced egg quality and/or production. Other factors that may affect egg quality and/or production were also examined.

Materials and methods

Data collection

Of the 31 layer farms contacted to take part in the study, 14 agreed to participate (17 flocks in total). Summary information for each farm is presented in Table 1. Flocks ranged in size from 2400 to 72,000 chickens per farm and were of various breeds.

Each farmer was sent 20 rayon-tipped dry swabs (MWE Medical Wire, Wiltshire, UK) 1–2 weeks prior to the required time of testing, with instructions to swab (trachea prior to lay/cloacal during lay) 20 randomly selected chickens immediately prior to IBV vaccination or approximately every 6–8 weeks. These swabs were then sent immediately by express mail to the laboratory for IBV testing.

It was requested that Haugh unit data for eggs that were laid on the same day as the swabs were collected be submitted with the swabs. The Haugh unit data were used to grade eggs as good or poor if the Haugh units were acceptable for the age of the hen based on standard management guides for Hy-line Brown, Isa Brown and Hisex layers.

Farmers were also asked to report throughout the study if any of the flocks were subjected to any stressors that could affect internal egg quality.

Analysis of submissions

Swabs were pooled into two groups of 10 and viral RNA extracted, cDNA synthesized and 3′ UTR PCR/HRM curve analysis performed as previously described.[19]

Submissions found to be positive or trace positive (indicating that virus was present but at levels insufficient for reliable HRM curve

Table 1. Profile of layer flocks participating in a study of the potential relationship between egg quality and infectious bronchitis virus infection

Flock ID	Farm location	Farm type	Breed	Flock size	Monitored age (weeks)	Vaccine used	Vaccination frequency[a]
1	NSW	Cage	Hy-line Brown	18,000	6–64	VicS & Vac-A	8
2	NSW	Free range	Hisex	14,000	6–46	VicS	Rearing only
3	NSW	Free range	Isa Brown	2400	23–42	I	6
4.1	QLD	Cage	Hy-line Brown	72,000	22–72	VicS	6
4.2	QLD	Cage	Isa Brown	72,000	18–68	VicS	6
5	SA	Breeders	Hy-line Brown	13,000	15–55	VicS & I	6
6	SA	Free range	Hy-line Brown	6000	13–82	VicS	8–10
7	TAS	Free range	Hy-line Brown	26,500	2–75	VicS	Rearing only
8.1	VIC	Cage	Isa Brown	65,000	9–95	I	6
8.2	VIC	Cage	Isa Brown	21,500	7–62	I	6
8.3	VIC	Cage	Isa Brown	21,000	20–64	I	6
9	VIC	Cage	Hy-line Brown	54000	18–75	I	6
10	VIC	Cage	Isa Brown	50,000	18–67	I	6
11	VIC	Cage	Isa Brown	51,000	7–45	I	6
12	VIC	Free range	Hisex	8000	19–72	I	6
13	VIC	Barn	Hisex	3500	36–45	I	6
14	VIC	Breeders	Hy-line Brown	35,000	19–45	I	8

[a]Number of weeks between vaccinations.
NSW, New South Wales; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria.

analysis) for IBV were subjected to 3′ UTR nucleotide sequence analysis as previously described.[35] Each nucleotide sequence was compared with sequences available publicly in the GenBank[37] database using a nucleotide BLAST search (blastn/megablast) against the 'others' database.

Statistical analysis

A mixed effects logistic regression, with a random effect of farm and a fixed effect of potential risk factor, was used to screen for potential risk factors that affect 'detection of IBV' (i.e. the factor was associated with an increased risk of IBV detection) and 'poor Haugh units' (i.e. the factor was associated with an increased risk of poor-quality eggs). A P-value <0.25 was used to identify potential risk factors that warranted further evaluation[38] when considering the effects on the flock of infection with IBV and/or producing eggs with poor Haugh units. Stata 11.1 software (StataCorp, College Station, TX, USA) was used for all analyses. The risk factors examined included flock age (≥20 or <20 weeks of age; reflecting whether the flock was in lay or not), breed of chicken (Isa Brown, Hy-line Brown or Hisex), type of farm (cage, free range or barn), flock size (≥15,000 or <15,000), whether the chickens were vaccinated or unvaccinated during lay and the frequency of vaccination (every 6 or 8 weeks, if vaccinated during lay).

Results

Detection of IBV

Layer flocks could be separated into three groups, based on detection of IBV using PCR/HRM curve analysis (Table 2). In 5 flocks, IBV was detected only once and at the low level as a trace positive. In 10 flocks, IBV was detected either once only as a positive result or ≤3 times as a combination of positive and/or trace positive results. In the other 2 flocks, IBV was detected ≥4 times as a combination of positive and/or trace positive results.

Strains of IBV

The IBV strains detected in 25 of the 37 (either positive or trace positive) submissions were characterised as V3/02-related strains using HRM curve analysis. Nucleotide sequencing of the 3′ UTR determined that each had the highest nucleotide sequence identity (>97%) with the V3/02 field strain. All these strains were considered field strains, as vaccine A was not used to vaccinate the flocks, with the exception of the strain detected in flock 1.

The IBV strains detected in five of the submissions were determined to be related to the subgroup 1 vaccines, VicS or I, using HRM curve analysis. Nucleotide sequencing confirmed these results (nucleotide sequence identity >99% with vaccines VicS and I, GenBank accession numbers DQ490221 and FJ235181, respectively). These detections were considered to be re-isolation of the vaccine/s used for flock vaccination.

Using HRM curve analysis and nucleotide sequencing of the 3′ UTR region, we determined that two submissions contained more than one strain of IBV. In submissions from flock 2, nucleotide sequencing indicated a mixed infection of V3/02 and a VicS-related strain (VicS was used to vaccinate the flock during rearing). In a submission from flock 5, nucleotide sequencing indicated a mixed infection of V3/02 and a variant field strain.

Table 2. Detection of infectious bronchitis virus (IBV) and strain/s identified in a study of the potential relationship between egg quality and IBV infection

Flock ID	Total no. of submissions	Positive (%)	Strain/s identified	Trace (%)	Strain/s identified
1	9	1 (11.1)	V3/02[b]	1 (11.1)	N/A[d]
2	4	2 (50.0)	I[a]/VicS + V3/02	1 (25.0)	V3/02
3	4	0 (0.0)	–	1 (25.0)	I[a]
4.1	8	1 (12.5)	V3/02	0 (0.0)	–
4.2	8	0 (0.0)	–	1 (12.5)	N/A
5	7	1 (14.3)	V3/02 + Variant[c]	1 (14.3)	N/A
6	10	1 (10.0)	V3/02	1 (10.0)	V3/02
7	13	0 (0.0)	–	1 (7.8)	VicS[a]
8.1	13	1 (7.7)	I[a]	2 (15.4)	N/A/V3/02
8.2	8	1 (12.5)	Variant	0 (0.0)	–
8.3	8	1 (12.5)	V3/02	6 (75.0)	V3/02
9	8	3 (37.5)	V3/02	2 (25.0)	N/A/V3/02
10	9	0 (0.0)	–	1 (11)	V3/02
11	7	0 (0.0)	–	2 (28.6)	V3/02
12	9	1 (11.1)	I[a]	1 (11.1)	V3/02
13	2	0 (0.0)	–	2 (100.0)	V3/02
14	3	0 (0.0)	–	1 (33.3)	Variant

[a]Strain identified as the vaccine used on-farm. [b]Strain identified as V3/02-related strain. [c]Strain identified as a variant field strain. [d]Nucleotide sequencing was unsuccessful.

IBV was detected once only from flocks 8.2 and 14 during their respective monitoring periods and HRM curve analysis and 3′ UTR nucleotide sequencing determined that both submissions contained variant strains of IBV (Table 2).

Nucleotide sequencing of the 3′ UTR determined that the variant strains detected from flocks 5 and 14 had highest nucleotide sequence identities with V3/02; however, they contained 175-bp and 189-bp deletions, respectively. The 3′ UTR of the variant strain detected in flock 8.2 had the highest sequence identity (>99%) with the subgroup 1 vaccines; however, its nucleotide sequence contained a 41-bp deletion (results not shown).

Haugh units

Haugh unit data were not collected from four of the flocks and in another two flocks visual observation on the farm reported the consistency of albumen as normal. The Haugh unit data collected for all other flocks are collated in Table 3.

Haugh units were acceptable for the age and breed of hen at the time of collection for all flocks of all ages except as shown. In 7% of cases, IBV was detected simultaneously with the production of eggs with poor Haugh units. All farmers reported that none of their flock was subjected to other stressors throughout the duration of the study that may have affected internal egg quality.

Table 3. Haugh unit data collected for each flock during the monitoring period in a study of the potential relationship between egg quality and infectious bronchitis virus infection

Flock ID	Breed	Age (weeks)	Within Haugh units range[a] (Y = yes/N = no)	IBV detected (Y = yes/N = no)
2	Hisex	22	NA	N
		46	Y	Y
4.1	Hy-line Brown	22, 28, 37, 55, 60, 72	Y	N
		43	Y	Y
		66	NA	N
4.2	Isa Brown	18, 33, 39, 51	Y	N
		24	Y	Y
		56, 68	N	N
		68	N	N
		62	NA	N
5	Hy-line Brown	20	NA	Y
		25	Y	Y
		28, 31, 45, 55	NA	N
6	Hy-line Brown	28, 38, 73	Y	N
		43, 54	N	Y
		48	N	N
		60, 67, 82	NA	N
8.1	Isa Brown	25, 65	Y	Y
		34, 41, 45, 54, 59, 69, 93, 95	Y	N
8.2	Isa Brown	21, 29, 35, 46	Y	N
		40	N	Y
		62	NA	N
8.3	Isa Brown	19	Y	N
		21, 64	NA	Y
		33, 39, 44, 49, 57	Y	Y
10	Isa Brown	18, 26, 32, 38, 45, 56, 62, 67	Y	N
		50	Y	Y
11	Isa Brown	22, 39	N	N
		27	NA	N
		35	Y	Y
		45	Y	N
13	Hisex	36, 45	N	Y

[a]Eggs were above the acceptable Haugh units for that particular breed of chicken at the age the Haugh units were collected. NA, data not received.

Potential risk factors for the detection of IBV in a flock

Using a mixed effects logistic regression analysis, a P-value >0.25 was used to identify potential risk factors for the detection of IBV in a flock. There was no potential association between any of the risk factors examined and the detection of IBV in a flock, including flock age (P = 0.62), breed of chicken (P = 0.27), farm type (P = 0.59), flock size (P = 0.40), whether the chickens were vaccinated during lay (P = 0.72) and vaccination frequency during lay (P = 0.53).

Potential risk factors for reduced Haugh units

Using a mixed effects logistic regression analysis, a P-value ≥0.25 was used to identify potential risk factors for a flock producing eggs with Haugh units below the accepted value indicated by the breeder. There was a potential association between the production of eggs with reduced Haugh units and the detection of IBV (P = 0.22), farm type (P = 0.22; this value excludes the data for barn-laying hens because of insufficient data for this farm type) and flock size (P = 0.09). There was no association between the production of eggs with reduced Haugh units and breed of chicken (P = 0.76), whether the chickens were vaccinated during lay (P = 0.88) and vaccination frequency during lay (P = 0.36). The breed Hisex was removed for this analysis because of insufficient data.

Discussion

The purpose of this investigation was to investigate the prevalence of IBV in Australian layer flocks and to assess whether the presence of IBV in a layer flock was related to reduced internal egg quality. Other factors that may result in the production of eggs with poor Haugh units were also examined. For the longitudinal study, 31 layer farms were contacted and 14 participated in this study, with 17 flocks in total sampled. The 14 farms that participated in this study were a good representative of the egg layer industry in Australia because they included cage, barn, breeder and free-range layers of three common breeds and the flocks varied in size between 72,000 to 2400 layers located in five States (New South Wales, South Australia, Tasmania, Victoria and Queensland). The duration of monitoring, and subsequently the number of samplings, varied for each farm, but all were monitored at some point during lay. Throughout the monitoring period, IBV was detected in all 17 layer flocks; in 5 flocks, IBV was detected only once and at a low level; in 10 flocks IBV was detected either once only as a positive or trace positive result; in 2 flocks, IBV was detected ≥4 times as a combination of positive and/or trace positive results.

Approximately two-thirds of the IBVs detected in this study were classified as V3/02-related field strains. All but three of the remaining IBVs detected were classified as subgroup 1 vaccine-related field strains or re-isolation of the vaccine/s used to vaccinate the flocks. The V3/02-related IBVs had the highest identities with the field strain V3/02, which was originally isolated in Victoria from broiler chickens with nephritis.[19,20] There have been a number of subsequent isolations of a V3/02-related field strain in our laboratory from broiler chickens (unpubl. data), despite the very limited use of vaccine A to vaccinate broiler chickens (Peter Scott, pers. comm.). Although IBV was detected in layer hens throughout this study, detection of IBV in a flock was not associated with clinical signs of disease, which potentially indicates recurrent subclinical infection because of some vaccinal immunity in the flock.[1]

Three of the IBVs detected were determined to be variant strains by nucleotide sequencing, because of the large deletions of varying sizes in the 3′ UTR. This is not surprising, because the variability of this region in IBVs has been widely reported.[19,20,39,40] The 3′ UTR nucleotide sequencing was not successful for the IBV present in five of the pooled submissions and we suspect that this is most likely related to a low level of viral RNA present in the original submissions.

It would have been beneficial to also sequence the S1 gene of each IBV detected, but our ability to produce this data was hampered by the varying quality of the RNA in the submissions. Sequencing of the S1 gene was also inhibited by the need to amplify the entire S1 gene (≈ 1600 bp) for comparison. Partial S1 gene sequencing is unreliable for strain characterisation because of the extensively reported ability of IBVs to recombine in the S1 gene region.[41–45]

For ease of sampling and processing, we decided that dry swabs would be more practical for the collection, storage and shipment of specimens by farmers, although it precluded isolation of live virus. The use of transport media to facilitate virus isolation was considered, but was thought to reduce the farmers' willingness to participate in this survey, because of the need to keep and ship the samples on ice, which would subsequently hinder the regularity of submissions and reduce the number of specimens collected. Upon arrival at the laboratory, however, the swabs were placed directly into lysis buffer for extraction of viral RNA and HRM curve analysis, and when a positive result was recorded, further swabs and/or tissue placed into transport media were requested. Numerous efforts were made throughout this study to obtain an isolate of IBV from layer flocks identified as IBV positive, but IBV could not be isolated from cloacal or oviduct swabs or directly from oviduct tissue of chickens after multiple passages through embryonated chicken eggs. The reasons for this are speculative, but may include that virus detected by PCR was potentially non-infectious (i.e. remnants of a previous infection) or in some cases present at a low level.

Virus isolation is the ideal methodology for investigating the potential effects of IBV infection on the production of eggs with poor internal quality; however, as described, virus isolation was unsuccessful in the present study. Serological analysis would not have provided useful data, because all flocks except for two were vaccinated multiple times to maintain high levels of IB immunity. Thus, although virus challenge might have occurred in immune hens, it would not have been detected in serological assays such as ELISA because there would be little detectable changes in the dominant cross-reactive antibodies. Only tests that detect IBV serotype-specific antibodies are useful for such purposes, and such tests are difficult and laborious. Therefore, PCR analysis, which is a simple and fast technique, was performed instead to detect the presence of viral RNA. This technique was successfully able to detect IBV RNA and hence identify IBV strains that were present in the hens at the time of sampling. The lack of recovery of an IBV isolate from layer chickens could also reflect the short duration and transitory nature of IBV infection, which is known to occur in immune hens.[22]

Various other factors besides IBV infection (e.g. nutritional deficiencies, concurrent disease challenge, hen age, egg storage temperature, time delay and humidity) can affect the Haugh unit measurement of an egg.[23–25,46,47] The timeline of experimental IBV infection and its relationship to when poor-quality eggs are detected indicates that a drop in egg production and egg shell quality becomes apparent during the second week after infection and may last for 2–4 weeks;[48] hence the effect occurs some time after virus has ceased replicating. How and when the Haugh units were measured for data collected on farm could not be controlled for this study, but based on discussions with each farmer, the data collected were assessed in line with standard procedures (Haugh units assessed against industry standards for hens' breed and age) to ensure the highest possible accuracy of the assessment of internal egg quality. Although these factors and inconsistencies lead to the conclusion that measurement of Haugh units is too subjective to be an effective measure of internal egg quality, measurement of the Haugh units, correlated with hen age, is the current industry standard for determination of egg quality. The majority of the Haugh unit data showed that all flocks produced eggs with above (in some cases well above) the average expected value for the respective breed of chicken at the age the swabs were taken.

The results demonstrated that the presence of IBV in a flock was not a causative agent of poor-quality eggs, as 76% of submissions that were positive for IBV were associated with Haugh units that were acceptable by industry standards for hen age and strain, although the statistical analysis indicated that the presence of IBV in a flock is a potential risk factor for the production of eggs with Haugh units that are outside the industry standards. This could suggest that potentially some Australian IBVs may have tropism for the oviduct, causing the production of poor-quality eggs with reduced albumen quality. Although in controlled studies some Australian IBV strains have been shown to grow in and damage oviductal tissues,[29,30] there has not been a successful isolation of a field strain of IBV from the oviduct of layer hens producing poor-quality eggs. Often other factors, besides infectious pathogens such as IBV, are involved in the production of poor-quality eggs.[21] Our study identified flock type as a potential risk factor; however, this risk factor is related to flock size (i.e. caged hens are housed at a higher density than free-range hens and therefore caged flocks are generally larger than free-range flocks). In terms of the effect of chicken breed on Haugh units, comparisons of Hy-line Brown, Hisex and Isa Brown hens have consistently demonstrated that eggs produced by Hisex and Isa Brown hens have lower Haugh units than eggs produced by other brown-egg layers.[29,47]

All farms practised varying IB vaccination protocols. In some cases, live vaccines I and VicS were used, but in other cases, live vaccines I, VicS and A were used in various combinations at different ages. Flocks were vaccinated every 6 or 8 weeks during lay and only two flocks were vaccinated during the rearing period only, despite a previous study that concluded there was little advantage and more disadvantage associated with regular revaccination during lay in Australian layer flocks.[22] The same study also indicated that effective vaccination during the rearing period was more important when considering IBV vaccination protocols.

International IB vaccination protocols include priming at an early age with a live IB vaccine followed by the use of inactivated vaccines (unavailable in Australia) prior to, and sometimes during, the lay period. This type of vaccination provides a more uniform level of immunity than live vaccines used periodically. Use of live vaccines in layers, as is currently practiced in Australia, is unconventional because it allows for possible reversion of vaccine virus to virulence and recirculation of the vaccine virus or vaccine-related strains. This appeared to be the case in the present study, considering almost all the strains were identified as vaccine or vaccine-related field strains. The regular use of live IBV vaccines throughout the life of a laying flock could potentially result in the emergence of new strains of IBV. This is supported by the results of a recent report that found viral subpopulations, with differing viral characteristics to the parent vaccine strain, in a commercial vaccine.[49] However, it is difficult to predict whether chickens will be protected against new strains of IBV, even those strains that appear to be highly related to vaccine strains, with the current vaccines.

This study identified that predominantly V3/02-related field strains are circulating in Australian layer flocks, including flocks situated on farms where vaccine A was not administered. Therefore, further investigation into the pathogenicity of V3/02-related field strains (such as V3/02) is recommended, to assess their particular risk to the egg industry. Lastly, the efficacy of the current vaccination protocols in prevention of infection of chickens with these IBVs needs to be assessed with regard to whether vaccine A should be used more prominently in layer hens to prevent infection with V3/02 field strains or whether the use of these vaccines on some farms is perpetuating the spread and persistence of V3/02 strains in commercial layer flocks.

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