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Variability in practices for drinking water vaccination of meat chickens against infectious laryngotracheitis

Peter J. Groves[A,*], Awol M. Assen[B], Ashley Etherington[C], Mark Stillman[D], Sheridan Alfirevich[D], Priscilla F. Gerber[B], Alex-Kate Langfield[E] and Stephen W. Walkden-Brown[B]

For full list of author affiliations and declarations see end of paper

*Correspondence to:
Peter J. Groves
Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, 425 Werombi Road, Camden, NSW 2570, Australia
Email: peter.groves@sydney.edu.au

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ABSTRACT

Context. Drinking water vaccination of young meat chickens with Infectious Laryngotracheitis (ILT) vaccine is problematic. Vaccine failure and adverse vaccine reactions are frequently reported. Variations in the technique of applying ILT vaccines by this mass vaccination method need to be understood to contribute to improving the success of vaccination. **Aims.** This study aimed to examine variations in the techniques of application of Infectious Laryngotracheitis vaccines via drinking water for young meat chickens. **Methods.** Drinking water vaccination techniques were observed and recorded across 52 broiler flocks during ILT outbreaks in three geographic areas of Australia. Descriptive statistics for all variables were computed and variations between integrator company procedures were statistically compared. **Key results.** Despite rigorous standard operating procedures, wide variations were observed in time of water deprivation prior to vaccination (3–15 min), time drinking water was stabilised prior to addition of vaccine and the type of stabiliser product used, time to activate the flock following filling of the water lines with vaccine (10–127 min), time for the vaccine to be consumed (36–226 min) and the volume of drinking water per bird used to provide the vaccine (11–48 mL/bird). **Conclusions.** Variation in vaccination technique can affect the success of drinking water vaccination against ILT in young meat chickens. **Implications.** Understanding the importance of the variable factors in vaccine application method can improve the success of water vaccination against ILT.

Keywords: broiler, chicken, drinking water, immunisation, infectious laryngotracheitis, poultry, poultry diseases, vaccination.

Introduction

Infectious laryngotracheitis (ILT) is a serious respiratory disease of chickens worldwide, caused by infection with an alphaherpesvirus (*Gallid alphaherpesvirus 1*). Most live attenuated vaccines against ILT are registered for use by individual eye drop administration or via drinking water (Hilbink *et al.* 1987; Coppo *et al.* 2012). ILT vaccination of flocks of commercial meat chickens is generally only envisaged in the face of a local outbreak and the huge numbers of birds involved requires the use of mass vaccination techniques, usually via drinking water at between 1 and 2 weeks of age (Coppo *et al.* 2012; Groves *et al.* 2019). Although registered for application by this method, drinking water application can produce variable results in terms of the effective proportion of birds that take up the vaccine virus initially (Groves *et al.* 2019). This is likely due to challenges in ensuring that sufficient amounts of the vaccine virus come into contact with respiratory tissues to actually vaccinate the bird (Hilbink *et al.* 1981; Robertson and Egerton 1981; De Wit 2013). Laboratory studies often show successful protection against challenge with field strains of ILT virus with the available vaccines (Arzey and Arzey 2009; Korsa *et al.* 2015) but problems in achieving protection in the field are commonly described (De Wit 2013; Keck 2018). A previous study (Groves *et al.* 2019) conducted in commercial meat chicken flocks in Australia demonstrated marked variation in vaccine virus establishment in respiratory tissues associated with drinking

water application factors. This previous study was limited in its ability to identify all the important administration factors as it included only eight flocks. During this and another subsequent study, an ability to estimate vaccine uptake success by quantitative polymerase chain reaction (qPCR) assay of dust samples was developed (Ahaduzzaman *et al.* 2020; Assen *et al.* 2020). Therefore, a larger field study involving 52 flocks across Australia was designed to look at the associations between variability in drinking water vaccine application and subsequent effectiveness of vaccination. The qPCR dust detection method (Ahaduzzaman *et al.* 2020) was used in this present field study but wild or vaccine strain ILT viral DNA was found to already be present in many flocks prior to vaccination (Assen *et al.* 2019). This compromised the ability to analyse the association of vaccination administration variables with vaccination success as virus may have been circulating in the flocks prior to vaccination. These associations will require further studies. Reported herein are the variations in drinking water vaccination techniques observed in this larger study of 52 flocks.

Companies provided specific ILT vaccination standard operating procedures (SOP) to farms involved in the current study, but nevertheless, substantial variations are thought to occur in application for a variety of reasons.

Materials and methods

Collaborators

Three regions of Australia were experiencing ILT outbreaks in meat chickens in 2018–2019. The companies farming in these areas were integrated operations, all operating hatcheries and abattoirs and using contracted farms to grow meat chickens. Two of these companies also operate their own breeding operations and feed mills. The integrator companies employ service personnel and veterinarians to provide supervision and advice to the contracted meat chicken growers. The companies supply chickens, feed and service; the contracted farmer provides facilities and labour. Either Cobb 500 or Ross 308 strain meat chickens were used. The service personnel from the companies supervised or performed the administration of ILT vaccines on the contracted farms, following a prescribed SOP. The companies also choose and supply the vaccine type to be used.

Vaccines

There are currently three attenuated, live chicken-embryo origin (CEO) vaccines available in Australia (García 2017; Fraser 2019). Two were developed in Australia (SA2 and A20 strains, Zoetis Poulvac Laryngo) and the third is imported (Serva strain, NOBILIS®ILT, MSD). Of these, only A20 and Serva strains are used in meat chickens as SA2, although genetically very similar to A20 (which was derived from SA2), is considered too pathogenic in this type of bird (Ou and Giambrone 2012). A20 and Serva strains are registered in Australia for use via drinking water (MSD undated; Zoetis undated). Strains SA2 and A20 are classified as Class 1 while Serva strain is designated as Class 7 using a restriction fragment frame length polymorphism (RFLP) technique (Kirkpatrick *et al.* 2006) which was subsequently modified to a multiplex polymerase chain reaction (PCR)-RFLP typing method (Williamson *et al.* 2019).

Procedures

Three different sites in Australia that were vaccinating commercial meat chickens against ILT were involved in the study. Two sites were in New South Wales: these were the greater Sydney basin, and a regional area in the Riverina district. The third site was in South Australia. Chickens were vaccinated using Serva strain in the greater Sydney region or A20 vaccine in the Riverina and South Australia.

Vaccination procedures followed SOPs according to each integrator company's requirements which were all closely based on guidelines specified by the vaccine manufacturers (MSD undated; Zoetis undated). Briefly these feature the following specifications:

- Vaccinate early in the day.
- Clean and rinse drinkers and avoid the presence of disinfectants in the drinking system.
- Adjust the water volume in the tank to the designated level using a formula to calculate required volume for vaccination based on the age and number of birds to provide water to be consumed within 1.5–2 h (volume (L) = the number of birds multiplied by their age in days multiplied by two). Where a medication tank is used, the volume is estimated in the tank. Some houses use automatic proportioners for provision of prepared vaccine directly into the water supply line. Typically, this method requires a water volume estimate (calculated as above or determined by measuring 2 h consumption the day before) and setting the proportioner to deliver the required volume of the prepared mixture of vaccine and water over that time.
- Withdrawal of drinking water from the birds for a specified time, either by shutting off the drinker lines or, more frequently, by raising the drinker lines out of reach of the birds.
- Adding a product to stabilise the water (i.e. to neutralise chlorine or salts that may inactivate the vaccine virus) such as skim milk powder (2.5 g/L) or a proprietary product containing a dye to protect the vaccine. A waiting time for stabilisation to occur is specified (commonly 20 min for skim milk but the proprietary dye products claim instant stabilisation).
- Preparing the vaccine in a small volume of stabilised water and then adding this to the medication tank

- Flushing the drinker lines with the water so that the skim milk or dye colour is seen at the end of the line to ensure vaccinated water is immediately available to all birds.
- Drive the birds towards the drinkers by walking through the flock.
- The vaccine mixture should be consumed within 2 h.

The target age for vaccination was between 7 and 14 days. Farms varied in their choice of stabiliser product, using skim milk powder (2.5 g/L water), liquid skim milk (approximately 17 mL/L water), or a proprietary stabiliser containing a blue dye: Vac-Pac Plus® (Animal Science Products Inc. undated) at 10 g/100 L drinking water; or DeCHLOR® (Feedwater undated) at 10 mL/100 L drinking water.

Measurements and records

Service personnel from the company supervised or conducted all vaccinations and then completed a detailed and standardised record sheet on the practices used. Descriptions of the house and procedures used were recorded, including flock size, proportion of the house available to the birds at the time of vaccination and number of drinker lines used, ventilation system, number of birds present, bird age at time of vaccination, vaccine strain used, and number of label doses delivered, and water volume used for vaccination. The duration of each procedure was recorded for time of water withdrawal, time at which stabiliser was added to the water supply, time at which vaccine was prepared, and time this was added to the drinker system, time that flushing of the lines to fill them with vaccinated water was completed, time that the staff walked through the house and time that vaccine was completely consumed.

Statistical analyses

All recorded data were entered into a computerised statistics package (Statistica v6.1, StatSoft Inc. 2003). Descriptive statistics were generated for each variable which consisted of the number of valid entries, means, standard deviation and coefficients of variation, the 95% confidence intervals of the mean, minimum, median, lower and upper quartiles, maximum values, skewness and kurtosis. Pearson correlation coefficients were calculated between quantitative variables. Comparison between practices in each company were compared using one-way Analysis of Variance with means separated using Tukey's HSD test. Where variables did not show homogeneity of variance (significant Brown–Forsythe test) then the non-parametric Kruskal–Wallis ANOVA was used. Results were considered significant at $P < 0.05$.

Animal ethics

The study was conducted under the supervision of the Animal Ethics Committee of the University of New England (authority number AEC19-011). All birds were held under normal commercial conditions within the operations of large integrated meat chicken companies and were subject to their animal welfare requirements and controls. Many of the farms used were Royal Society for Prevention of Cruelty to Animals (RSPCA)-accredited establishments. This was an observational study only; no experimental interventions were performed.

Results

Table 1 shows qualitative factors that were fixed for the farm at the time of vaccination (i.e. location, integrator company, house design, strain of chicken supplied, hatchery supplying chicks and hatchery vaccinations applied). The strain of ILT virus (ILTV) vaccine used is also chosen by the integrator company for the location of the farms.

A variety of descriptive statistics for quantitative variables are displayed in Table 2 (number of flocks supplying data, mean value and 95% confidence intervals for the mean, minimum value, upper and lower quartile, median and maximum values, standard deviation, coefficient of variation, skewness and kurtosis). The majority of the data distributions were moderately positively skewed (skewness greater than

Table 1. Qualitative data – fixed factors in the vaccination database.

Factor	No. of flocks in each category			
Company	A	B	C	
Flocks	20	12	20	
Growing region	NSW	SA		
Flocks	32	20		
Flock ventilation design	Conventional	Free range	Tunnel ventilated	
Flocks	14	4	34	
Breed	Cobb	Ross		
Flocks	28	24		
ILTV vaccine strain	Serva	A20		
Flocks	28	24		
Stabiliser used	Skim milk	Proprietary dye	Skim milk + dye	
Flocks	14	18	20	
Hatchery	A	B	C	D
Flocks	16	12	4	20
Hatchery vaccinations	IB only	IB and ND	IB, ND and MD	
Flocks	14	31	3	
Litter age (batches)	1	2	4	
Flocks	39	3	1	

IB, infectious bronchitis vaccine; ND, Newcastle disease vaccine; MD, Marek's disease vaccine.

+0.5). Thus, most of the values in the distributions are less than the mean, the mean being elevated by a few very high values. All of the distributions are platykurtic (Kurtosis <3.0), as the values towards the extremities are less than would be expected in a normal distribution (Dugar 2018). Table 2 also shows the ranges and variation in the recorded variables across the 52 flocks in the study. The factors involved in the practice of vaccination for ILT showed marked variation with coefficients of variation for the time observations ranging from 39.2 to 95.4% (Table 2). The key variables of concern are noted below.

- The length of water deprivation prior to vaccination ranged between 3 and 145 min with a median time of 42 min.
- Time of stabilisation of the drinking water ranged from 0 to 118 min with a median time of 5 min. This would reflect the choice of stabiliser, with skim milk requiring 20 min but the proprietary dye products claiming instant stabilisation. Thirty flocks (58%) had a stabilisation time between 0 and 20 min.
- Time from the start of vaccine availability until the birds were activated by staff walking the house ranged from 10 to 127 min with a median of 22.5 min. One operator walked the flock prior to vaccination beginning and the task was completed in 19 flocks (37%) within 20 min of vaccine availability to the birds.
- Time to consume the vaccine varied from 36 to 226 min with a median time of 104 min. This is within the target time of <120 min according to the SOPs. This may have been affected by when the farmer deemed the process 'finished'. Some tanks were empty as soon as the drinker lines were flushed, while others took some time to empty.
- Nearly all birds were vaccinated between 7 and 13 days of age but one flock was not vaccinated until 18 days. The proportion of the house in use at the time of vaccination varied from 26.7% to the full house.
- The volume of water used to vaccinate varied between 11.3 and 47.9 mL per bird. This was confounded by company and by the variation in age of bird vaccinated across the sampled population.
- Delivery of a full label dose is a recommendation of the manufacturer with vaccines registered for drinking water delivery (APVMA undated; Zoetis undated). The actual number of doses applied (as specified on the label) depended on the vial size (either 2000 or 5000 doses per vial) and the actual number of birds present. The distribution of values of the number of label doses of vaccine supplied per bird was strongly negatively skewed (skewness = −1.47) illustrating the understandable tendency of the administrators to slightly overdose rather than underdose.

Table 3 is a rectangular matrix displaying Pearson coefficients of correlation between quantitative variables. Table 3 displays 68 individual correlation coefficients and hence, by definition, at least three to four of these could have shown significance by chance alone. Many of the coefficients were statistically significant but most were weak correlations ($-0.5 < r < +0.5$). Only the variables of age of ILT vaccination, time of day that vaccine preparation began, the time from vaccine being available to the birds until they were activated by staff walking through the flock, and the time to consume vaccine were normally distributed (Kolmogorov–Smirnov and Lilliefors tests of normality $P > 0.05$ – data not shown), hence some correlations may be unreliable with other variables.

Age of the birds at vaccination was positively correlated to flock size variables (farm size and number of birds per flock) which would indicate that larger farms tended to be vaccinated at slightly older ages.

The time allowed for the drinking water to be stabilised was weakly negatively correlated to larger farm and flock size variables, and to bird age and the time after vaccine availability that the birds were activated. This may indicate that staff were more hurried on larger farms. This is supported by the significant but weak positive correlation of stabilisation time with water deprivation time (i.e. shorter stabilisation time was associated with shorter water deprivation times). Stabilisation time was also negatively associated with time of day that it was conducted, indicating shorter stabilisation times as the day proceeded, again possibly a factor of flock size (taking longer to vaccinate a larger farm). The time of day that vaccination began (as evidenced by the time when vaccine stabiliser was added to the water) was moderately positively correlated ($r = 0.67$) with the time after vaccine was made available that the farmer walked through the flock, activating the birds. This may also be associated with larger farms, as busier staff may take longer to access the flocks.

The time between preparation of the vaccine (in a small volume of water to be added to the total volume) was weakly negatively correlated with the time that staff walked through the flock to activate the birds to drink after vaccine was available to the birds ($r = -0.49$) and the time for the birds to consume the vaccine ($r = -0.42$).

Table 4 displays comparative practices between the three meat chicken companies that participated in the studies. Despite very similar SOPs for ILT drinking water vaccination, the details of their practices differed significantly in many aspects. A major contributing factor here was comparative farm and flock size, with company C having very large houses and farms with more flocks, company B being much smaller and company A being between these extremes. The studies were also conducted at different times, with companies A and B studied in late 2018 to early 2019 and company C being involved later in 2019. Average age of application of the vaccine was around 10 days for companies A and B but tended to be older for company C (about 14 days). The size of the flocks dictated the number of drinkers in use at time of vaccination and the total

Table 2. Descriptive statistics of vaccination procedures recorded from 52 flocks in the study.

Vaccination data or procedure	Valid *N*	Mean	95% Confidence limits of the mean		Minimum	Lower quartile	Median	Upper quartile	Maximum	s.d.	CV%	Skewness	Kurtosis
			Lower	Upper									
Total birds on farm	52	256 100	208 378.2	303 821	53 500	111 000	243 840	346 500	581 853	171 412	66.9	0.70	−0.78
No. of flocks on farm	52	7	6.1	7.9	2.0	5	6	12	12	3.3	47.1	0.62	−1.06
Age of ILT vaccination (days)	52	11.4	10.6	12.1	7.0	10.0	11.0	13.0	18.0	2.6	22.8	0.40	−0.12
No. of birds at vaccination per flock	52	32 482	29 316.6	35 648.0	14 128	21 257	38 491	40 351	48 313	11 371	35.0	−0.34	−1.43
No. of drinker lines used for vaccination	49	4.8	4.6	5.0	3	4	5	5	6	0.76	15.8	0.07	−0.68
Proportion of flock in use at time of vaccination (%)	44	81.9	75.0	88.9	26.7	68.6	100.0	100.0	100	22.9	28.0	−0.91	−0.44
Time birds off water prior to vaccination (min)	52	49.7	39.7	59.6	3.0	21.5	42.0	69.5	145.0	35.8	72.0	0.87	0.37
Tank stabilisation time (min)	52	18.9	11.7	26.2	0.0	2.5	5.0	41.5	118.0	26.1	138.0	1.66	2.65
Time from vaccine preparation until available (min)	52	20.9	17.8	24.1	5.0	12.0	19.0	27.5	50.0	11.3	54.0	0.64	−0.07
Time from vaccination start until flock walked (min)	40	37.3	25.9	48.7	−10.0	6.5	22.5	70.0	127.0	35.6	95.4	0.74	−0.54
Time to consume vaccine (min)	43	109.1	95.9	122.3	36.0	82.0	104.0	127.0	226.0	42.8	39.2	0.64	0.51
Label vaccine doses supplied/bird	48	1.04	1.02	1.06	0.83	1.03	1.05	1.08	1.14	0.07	6.7	−1.47	2.19
Water volume for vaccination (mL/bird)	51	25.4	23.0	27.8	11.3	21.0	25.0	31.3	47.9	8.49	33.4	0.78	0.66

s.d., standard deviation; CV%, coefficient of variation %.

Table 3. Pearson correlation coefficients (*r*) between various observations of ILT vaccination procedure on 52 flocks.

Observation	Pearson correlation coefficient (*r*)							
	Bird age at ILT vaccination (days)	Water deprivation time (min)	Tank stabilisation time (min)	Time from vaccine preparation to available (min)	Time after vaccine available that birds were activated (min)	Time to consume (min)	Label doses/ bird	Water volume for vaccine (mL/bird)
No. of flocks on farm	**0.44***	**−0.31***	**−0.46***	**0.44***	−0.09	−0.21	0.10	**0.31***
Total birds on farm	**0.41***	−0.13	**−0.44***	**0.34***	0.03	−0.13	0.02	**0.32***
No. of birds in flock at vaccination	**0.32***	0.19	**−0.44***	0.08	**0.33***	0.26	−0.07	0.02
Water volume used for vaccination (L)	**0.56***	0.03	**−0.30***	**0.32***	−0.16	−0.12	−0.21	**0.66***
Time of day stabiliser prepared	0.06	**−0.44***	**−0.55***	0.14	**0.67***	**0.44***	0.34	−0.20
Bird age at ILT vaccination (days)		0.13	**−0.45***	−0.23	**0.38***	0.19	−0.12	**0.39***
Water deprivation time (min)	0.13		**0.35***	−0.49	0.15	**0.39***	−0.22	0.22
Tank stabilisation time (min)	**−0.45***	**0.35***		0.02	**−0.54***	−0.25	−0.12	0.16
Time from vaccine preparation to available (min)	−0.23	**−0.49***	0.02		**−0.49***	**−0.42***	−0.01	0.06
Time after vaccine available that birds were activated (min)	**0.38***	0.15	**−0.54***	**−0.49***		**0.35***	0.12	−0.17
Time to consume (min)	0.19	**0.39***	−0.25	**−0.42***	**0.35***		−0.10	−0.22
Label doses/bird administered	−0.12	−0.22	−0.12	−0.01	0.12	−0.10		**−0.37***
Water volume for vaccine (mL/bird)	**0.39***	0.22	0.16	0.06	−0.17	−0.22	**−0.37***	

*Coefficients highlighted in bold type are statistically significant ($P < 0.05$).

volume of drinking water used for each flock. Age of vaccination would also have been a factor in water volume used. The proportion of the house available for the chicks also varied with company management style with company C using the full house while the other companies had restriction of amount of space utilised (65–83% in companies A and B). Company A used a much shorter period of water deprivation prior to vaccination than did companies B and C (22 min compared to 69–65 min respectively). Time allowed for the water to be stabilised prior to the addition of vaccine varied markedly with company C averaging only 3.4 min, as did the time from vaccine preparation until its presentation to the birds, but this was probably due to this operation using proportioners to dose water rather than a medication header tank. Company A also had shorter stabilisation time (11 min) than company B (58 min) but this reflects the choice of stabiliser where the proprietary dye does not require a lengthy time compared with skim milk products. Company B personnel walked through the flock earlier following vaccination application than either companies A or C. Time to consume the vaccine was not significantly different between companies, generally taking between 1.5 and 2 hours. The amount of water used per bird to supply the vaccine varied but may have been confounded by the bird age at the time for company C compared to company A. Company B used a higher water allocation than company A despite similar bird age. The actual vaccine supplied per bird was close to one label dose although company A seemed likely to oversupply slightly, but significantly, compared to the other two companies.

Discussion

The wild strain of ILT virus causing the outbreak in the greater Sydney region was identified as Class 9 (Fraser 2019), which had been the predominant strain in Australia since 2009 (Agnew-Crumpton *et al.* 2016). However, the outbreak strain in the Riverina and in South Australia was identified

Table 4. Variation in ILT vaccination practices between companies.

Variable	Company A (*N* = 20) Mean (95% confidence interval)	Company B (*N* = 12) Mean (95% confidence interval)	Company C (*N* = 20) Mean (95% confidence interval)	*P*=[A]
No. of flocks on farm	7.3A (5.7–8.9)	4.2B (3.1–5.2)	8.4A (7.0–9.8)	0.0009
Total birds on farm	246 501A,B (161 713–331 288)	115 433B (46 377–184 490)	350 098A (288 516–411 680)	0.003
Date of ILT vaccination	12/01/2019	18/12/2018	29/08/2019	0.004[B]
Age of birds at ILT vaccination (days)	9.6B (8.8–10.4)	10.1B (9.1–11.0)	13.9A (13.1–14.7)	<0.0001
No. of birds in flock at ILT vaccination	30 523B (25 084–35 962)	23 172B (15 189–31 115)	40 028A (39 383–40 673)	0.0006[B]
No. of drinker lines used at vaccination	4.8A (4.4–5.3)	4.0B (3.7–4.3)	5.2A (5.0–5.4)	0.0001[B]
No. of bays available to chicks at vaccination	23.8A,B (16.7–30.9)	19.5B (15.8–23.2)	35.0A (30.5–39.5)	0.003[B]
Percentage of house in use at vaccination	65.1B (53.0–77.2)	83.3B (72.3–94.2)	100A (100–100)	<0.0001[B]
Time of water deprivation prior to vaccination (min)	22.4B (12.9–31.9)	69.5A (54.4–84.6)	65.0A (47.4–82.6)	<0.0001
Time drinking water stabilised (min)	11.2B (2.9–19.5)	57.67A (44.8–70.5)	3.4B (2.0–4.8)	<0.0001
Time from vaccine preparation until available (min)	27.7A (22.0–33.3)	20.4A,B (15.7–25.1)	14.5B (10.4–18.5)	0.005
Time from vaccination until flock walked (min)	33.8B (14.0–53.5)	6.5C (4.0–9.0)	72.8A (64.2–81.3)	<0.0001
Time to consume vaccine (min)	97.6 (62.7–132.5)	95.0 (81.2–101.8)	126.8 (109.0–144.7)	0.066
Water volume used for vaccination (L)	621B (430–812)	583B (463–703)	1412A (1258–1567)	<0.0001
Water volume for vaccine per bird (mL/bird)	19.5B (16.7–22.3)	28.2A,B (21.4–35.1)	29.2A (26.4–32.0)	0.0003
Label doses of vaccine supplied per bird	1.08A (1.06–1.10)	1.00B (0.65–1.05)	1.03B (0.99–1.06)	0.006

A, B, C, Means within a row with different letters differ significantly ($P < 0.05$).
[A]Probability difference due to chance (ANOVA separated by Tukey's HSD unless otherwise specified).
[B]Probability difference due to chance (Kruskal–Wallis test if variance non-homogeneous).

as Class 7 (Fraser 2019; Williamson *et al.* 2019) which may be a recombinant strain derived from the Serva vaccine which was subsequently identified as Class 7b by whole genome analysis (Sabir *et al.* 2020). The A20 vaccine strain was in use in the Riverina region of NSW and in South Australia while Serva vaccine strain was used in the greater Sydney region of NSW. In many of the flocks in the study, ILTV DNA of Classes 7 and 9 were detected in dust samples from the houses prior to vaccination being administered in the region being vaccinated with Serva strain, and from Class 7 in the regions vaccinated with A20 strain (Assen *et al.* 2019). It is not known whether the Class 7 detections were actually Class 7b (Sabir *et al.* 2020) as this nomenclature was not recognised at the time of testing.

The ILT vaccine manufacturers specify that a full label dose must be delivered per bird for effectiveness (MSD undated; Zoetis undated). However mass administration techniques do not guarantee that the complete designed dose will actually reach the respiratory target tissues. It has also been shown that it may require at least a ten-fold higher virus dose for drinking water application to achieve a similar effect to a single dose via individual eye drop (De Wit 2013). The ability of ILT vaccine virus to contact respiratory tissue (conjunctiva, nasal mucosa, inner choanae, larynx or trachea) is imperative for effective vaccination to occur (Robertson and Egerton 1981) but this is highly variable between birds using mass administration (Groves *et al.* 2019). Mass vaccination via drinking water application provides variable outcomes in this respect (Coppo *et al.* 2012) and relies extensively on bird to bird spread following successful initial vaccine uptake by only a proportion of the flock (Groves *et al.* 2019). Some of this wide variation in initial vaccine uptake may perhaps be due to subtle variations in the drinking water administration technique. The present study has shown that many variations in details of the vaccine administration method may occur in spite of rigorous SOP instructions. Many significant variations in process were observed between companies, as evidenced by the large coefficients of variation in all procedures, much of which was due to differences in farm and flock size, the method of water dosing (medication tanks compared to proportioners), differences in age that birds were vaccinated and choice of water stabilisation product. It has previously been shown that the proportion of birds taking up the vaccine quickly following vaccination can be affected by the application method and also by the stabiliser used (Groves *et al.* 2019; Assen *et al.* 2020) and this can affect the adequacy of vaccine protection and the occurrence of vaccine reactions. Hence the extent of variations in these techniques can have major effects on

vaccination success. Further studies need to focus on the actual contributions of the various application factors on the uptake of the vaccine by birds at the time of administration.

It was unfortunate that the detection of the presence of ILTV DNA in dust prior to vaccination on many farms eliminated the ability of the study to make associations between variation in administration technique and subsequent vaccine uptake by the birds. Further studies to understand the association of drinking water vaccination practices with ILT vaccination success are needed where vaccine uptake can be assessed without complication from unintended presence of virus (either wild or vaccine strains) prior to vaccine administration. The present study detected the presence of extraneous virus on the day of vaccination using environmental dust samples (Ahaduzzaman *et al*. 2020; Assen *et al*. 2020). Collection of individual bird samples such as tracheal swabs or feather Davidson *et al*. (2018) may have provided additional insight but would have required a significantly greater number of samples and, if the chickens were also positive for ILTV prior to vaccination, would not have overcome the problem of determining vaccination success in chickens already infected with ILTV. Indeed, we have subsequently shown that many flocks with positive dust samples prior to vaccination harbour active infection with ILTV as determined by qPCR of tracheal swabs (Assen *et al*. 2022). Studies on ILTV detection in feather shaft have occurred in older layer chickens, and the delay in time of detection using this method following vaccination may limit the value of this in young broilers where an assessment of vaccine uptake within 4–7 days is essential. Further studies to understand the most important factors involved in achieving a better initial flock uptake of the vaccine virus will lead to more efficacious field vaccination.

Conclusions

Even when an SOP is followed, variation in vaccination practices with ILT vaccines via drinking water shows marked flock to flock variation. The variations, for both fixed and variable factors, need to be assessed for associations with an accurate estimate of effective vaccine 'take' in each flock if the complication of an existing circulating ILT virus before vaccination can be understood and controlled.

This will assist in optimising ILT vaccination in future.

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Author affiliations

[A]Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, 425 Werombi Road, Camden, NSW 2570, Australia.

[B]School of Environmental and Rural Science, The University of New England, Armidale, NSW, Australia.

[C]Ingham's Enterprises Pty Ltd, Burton, SA 5110, Australia.

[D]Baiada Farms Pty Limited, NSW, Pendle Hill, NSW 2145, Australia.

[E]Zootechny Pty Ltd, Austral, NSW 2179, Australia.