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Investigations into a novel recombinant vaccine to control infectious laryngotracheitis virus

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Project No: 09-06 UoM (Devlin)

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In submitting this report, the researcher has agreed to the Australian Poultry CRC publishing this material in its edited form.

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Executive summary

Infectious laryngotracheitis (ILT) is an acute respiratory disease of poultry that is caused by infection with infectious laryngotracheitis virus (ILTV). This alphaherpesvirus is a significant concern for the poultry industry in Australia and overseas. To enhance control of ILT we have generated a novel candidate vaccine strain of ILTV that is deficient in the virulence factor glycoprotein G (gG). Benefits of this vaccine, over conventionally attenuated ILT vaccines, include a high level of vaccine safety and the potential to serologically differentiate between infected and vaccinated birds. In this project this gG-deficient vaccine strain of ILTV was studied in order to determine its transmission characteristics and to compare the safety and efficacy of this candidate vaccine with conventionally attenuated ILTV vaccines.

In the first component of this project the horizontal transmission of gG-deficient ILTV in naïve, specific-pathogen-free chickens was characterised and the basic reproductive rate was calculated. The horizontal transmission of the parental, virulent (wildtype) strain of ILTV was similarly characterised. Finally the transmission of wildtype ILTV in birds vaccinated with gG-deficient ILTV was studied. The results showed that both wildtype ILTV and gG-deficient ILTV were able to spread horizontally. Vaccine efficacy calculations revealed that the vaccine was successful in preventing 77% of cases of transmission of wt ILTV over one transmission cycle. The effective reproductive rate (R) of wildtype ILTV in vaccinated birds was < 1. This indicates that infection with virulent virus would be eliminated in a population of birds vaccinated with gG-deficient ILTV. Additional findings showed that the attenuated phenotype of gG-deficient ILTV was stable following one *in vivo* passage and subsequent natural infection of the in-contact birds. The findings from this component of the project point to the suitability of gG-deficient ILTV for use as a vaccine to control ILT under conditions that simulated natural exposure to virulent virus.

In the second component of this project gG-deficient ILTV was compared to conventionally attenuated vaccine strains of ILTV. Vaccine safety was assessed by comparing weight gain and clinical signs of disease in chickens vaccinated with different ILTV vaccines. Vaccine efficacy was assessed by comparing weight gain, clinical signs of disease and tracheal pathology in vaccinated birds following challenge with virulent virus. The findings from this component of the project show that gG-deficient ILTV has a high level of vaccine safety and a similar level of vaccine efficacy compared to other ILTV vaccines under experimental conditions that utilised intra-tracheal challenge of virulent virus.

The findings from this project support the continued development of gG-deficient ILTV as an attenuated vaccine for use in Australian and international poultry industries. This gG-deficient ILTV vaccine has the potential to improve control of ILT in Australian poultry. Benefits of this vaccine, over conventionally attenuated vaccines, include enhanced vaccine safety and the potential to serologically differentiate vaccinated birds from infected birds.

To take full advantage of the high level of safety of gG-deficient ILTV it is recommended that future studies commence with an investigation into administering higher doses of this virus at vaccination (a dose-response study). This has not been investigated previously. It is likely that higher levels of vaccine efficacy may be achieved by optimising the vaccine dose. It is also recommended that future studies into the continued development of this vaccine be conducted in conjunction with studies to develop a differential ELISA to discriminate between vaccinated and infected birds (based on the absence or presence of serum antibodies against gG). This would be beneficial for use in ILT eradication programs.

Contents

Introduction

Infectious laryngotracheitis (ILT) is an acute respiratory disease of poultry that is caused by infection with infectious laryngotracheitis virus (ILTV) [1]. This alphaherpesvirus is a significant concern for the poultry industry in Australia and overseas. To enhance control of ILT we have generated a novel candidate vaccine strain of ILTV that is deficient in the virulence factor glycoprotein G [2]. Glycoprotein G (gG) is a viral chemokine binding protein [3]. Viral chemokine binding proteins are an immune evasion strategy that large DNA viruses, like herpesviruses, use to help them survive in their hosts and cause disease [4]. This gG-deficient strain of ILTV (ΔgG ILTV) is significantly attenuated compared to the parent wildtype virus and has a high degree of safety and vaccine efficacy when delivered by eye-drop or drinking water. Benefits of this candidate vaccine, over conventionally attenuated ILTV vaccines, include an increased level of safety and the potential to serologically differentiate between vaccinated and infected birds [2, 5].

Objectives

This strategic project aimed to further investigate the suitability of this vaccine for use in chickens. The first component of this project aimed to investigate the horizontal transmission dynamics of ILTV to determine if vaccination with ΔgG ILTV induces sufficient flock immunity to prevent spread of wildtype virus, and also to determine if ΔgG ILTV can be transmitted horizontally between birds. Furthermore this study aimed to assess the stability of the attenuated phenotype of ΔgG ILTV over one *in vivo* passage.

The second component of this project aimed to directly compare this novel candidate vaccine strain with the three commercial ILT vaccines that are currently used in Australia. This study aimed to compare the different vaccines with regards to safety, efficacy and latency. This study also aimed to provide serum samples to facilitate the future development and testing of a differential ELISA to discriminate between birds vaccinated with ΔgG ILTV and field strains of ILTV.

Methodology

Methodologies utilized in the transmission study

Virus strains and propagation

The construction of a gG-deletion mutant of ILTV (ΔgG ILTV) from the virulent wildtype CSW-1 strain (wt ILTV) has been described previously [6]. All virus strains were propagated in the chicken hepatoma cell line LMH [7] as previously described [8].

Infection of SPF chickens with ILTV by eye-drop

Specific pathogen free chickens were obtained from SPFAS Australia Pty Ltd. Birds were individually identified using numbered wing-tags. At approximately 5 weeks of age the birds were randomly allocated into 2 groups and were inoculated by eye-drop with 4,500 plaque forming units (PFU) of ΔgG ILTV or wt ILTV suspended in 30 µl of media. Each group of birds was housed in a separate isolator unit and provided with food and water *ad libitum*. Three days after inoculation, swabs of the conjunctiva and trachea were collected from each bird to test for the presence of ILTV DNA and thus confirm ILTV infection. Four days after inoculation, birds infected with wt or ΔgG ILTV were transferred to new (clean) isolators housing naïve or vaccinated in-contact birds in order to study the transmission of infection.

Transmission of wt ILTV in naïve chickens

To study the transmission of wt ILTV in naïve chickens, 1 wt ILTV-infected chicken was co-housed with 10 naïve SPF chickens. This was performed in triplicate. Each of the 3 replicates (consisting of 1 wt ILTV-infected bird and 10 naïve birds) was housed in a separate isolator unit and provided with food and water *ad libitum*. All birds were approximately 6 weeks old at the beginning of the transmission study and were individually identified using numbered wing tags. The weight of each naïve bird, immediately prior to the addition of the infected birds, was recorded. Four days after the addition of the infected birds, conjunctival and tracheal swabs were collected from each in-contact bird to test for the presence of ILTV DNA. Six days after the addition of the infected birds, all the birds were culled by exposure to halothane and their weights were recorded. Conjunctival swabs and tracheal scrapings were collected from each of the in-contact birds during necropsy to test for the presence of ILTV DNA.

Transmission of ΔgG ILTV in naïve chickens

To study the transmission of ΔgG ILTV in naïve birds, 1 ΔgG ILTV-infected chicken was co-housed with 10 naïve SPF chickens. This was performed in triplicate. All chickens were approximately 6 weeks old at the beginning of the study. The chickens were housed and identified as described above. Samples were collected from the conjunctiva and trachea of the in-contact birds to assess the transmission of ILTV as described above. The weight of the in-contact birds was also recorded as described above.

Transmission of wt ILTV in chickens vaccinated with ΔgG ILTV

To study the transmission of wt ILTV in birds vaccinated with ΔgG ILTV, 1 wt ILTV-infected chicken was co-housed with 10 vaccinated SPF chickens. This was performed in triplicate. Each of the vaccinated birds had received 3,000 PFU of ΔgG ILTV suspended in 30 µl of media 3 weeks prior to the start of the transmission study. The birds were 3 weeks old at the time of vaccination. The vaccine was administered by eye-drop and the birds were subsequently housed together in one isolator unit and provided with food and water *ad libitum.* Three new (clean) isolators were each populated with 10 of these vaccinated birds for the transmission study. Birds were provided with food and water *ad libitum* during the transmission study and identified with numbered wing tags. Samples from the conjunctiva and trachea of the in-contact (vaccinated) birds were collected to assess the transmission of wt ILTV as described above. The weight of the in-contact birds was also recorded as described above.

Detection of ILTV DNA in conjunctival or tracheal samples

Swabs collected from the conjunctiva or trachea of the birds were immediately placed in 500 µl of Dulbecco's Minimal Essential Medium (Sigma) supplemented with 1% v/v foetal bovine serum (CSL), 10 mM HEPES pH 7.6 and 50 µg ampicillin /mL. Tracheal scrapings collected at post-mortem were similarly transferred to 500 µl of media. To extract DNA from these samples, 200 µl of the sample was collected and DNA was extracted using VX Universal Liquid Sample DNA Extraction Kit (Qiagen) and a Corbett X-tractor Gene Robot (Corbett Robotics, Australia). A total of 410 samples were prepared for DNA extraction from the ILTV-inoculated or in-contact birds. Each 96-well plate loaded onto the robot for DNA extraction had at least 10 negative extraction control wells containing sterile media only. These negative extraction control wells were distributed across different rows and columns of the plate. Partially full plates had at least one negative extraction control well per column. All plates had 1 positive extraction control well containing stock material of ILTV grown in cultured cells. Following DNA extraction, qPCR was performed as previously described [6] to assess if ILTV DNA was present in the extracted sample. Each sample was assayed once. A sample was determined to be positive for the presence of ILTV DNA if the copy threshold (ct) value was below 35 and if the dissociation curve was consistent with those from positive control samples.

The case definition of an infected bird was a bird that returned any sample (conjunctival and/or tracheal) that was positive for the presence of ILTV DNA at any time point (4 and/or 6 days post-contact). This case definition was developed to accommodate variation in the progression of infection between individual birds. Birds that did not meet these criteria were recorded as not infected.

Data analysis

The reproductive rate of ILTV infection in each of the experimental groups was determined. Measures of association were calculated to assess the effect of vaccination on the transmission of wt ILTV, as well as the association between infection with different strains of ILTV and the site where ILTV DNA was detected. The percentage weight gain in different categories of in-contact birds was analysed as a measure of virus virulence. Weight gain was compared using a student t test.

Methodologies utilized in the vaccine study

Virus strains

This component of the project utilized the same gG-deletion mutant of ILTV (ΔgG ILTV) [6]. This virus was propagated in the chicken hepatoma cell line LMH [7] as previously described [8]. The three commercial vaccines utilised in these studies were SA2 ILTV (Fort Dodge), A20 ILTV (Fort Dodge) and Serva ILTV (Intervet). A virulent isolate of CSW-1 ILTV was used to challenge the vaccinated birds.

Assessing vaccine safety

Groups of 20 SPF chickens at three weeks of age were vaccinated with A20 ILTV, SA2 ILTV, Serva ILTV or ΔgG ILTV, or remained as unvaccinated controls. Each group of birds was housed in a separate isolator unit and provided with food and water *ad libitum*. All birds were individually identified using numbered wing tags. The weight of each bird prior to vaccination was recorded. Each of the commercial vaccines was administered by eye-drop according to manufacturers' instructions. Each of the birds vaccinated with ΔgG ILTV received 3,000 PFU of ΔgG ILTV suspended in 30 μl of media by eye-drop. Clinical signs of disease were assessed and scored 5 days after vaccination. Demeanour was scored 0 (normal), 1 (depressed) or 2 (severely depressed). Dyspnoea was scored 0 (normal), 1 (dyspnoea with beak closed), 2 (dyspnoea with beak open), 3 (gasping) or 4 (severe gasping). Conjunctivitis was scored 0 (normal), 1 (partial closure of eye) or 2 (complete closure of eye). Clinical scores were summed for each bird to give an overall clinical score. Results were compared between groups using a Mann-Whitney test. The weight of each bird 21 days after vaccination was recorded. The weight of each bird, relative to the weight of that bird prior to vaccination, was calculated as a measure of vaccine safety. Results were compared between groups using a student t test. Serum was collected from vaccinated bird 21 days after vaccination and stored for future use.

Assessing vaccine efficacy

Twenty-one days after vaccination, each bird was challenged with 4,500 PFU of virulent ILTV administered intra-tracheally in 300 μl of media. Clinical signs of disease were scored 5 days after challenge as described above. Results were compared between groups using a Mann-Whitney test. All birds were culled 6 days after challenge. The weight and sex of each bird was recorded. Bird weights were compared between groups using a student t test. Transverse sections of proximal trachea were collected and prepared for histological analysis and lesion scoring as previously described [6]. Histological scores were compared between groups using a Mann-Whitney test. Scrapings of tracheal mucosa were collected and transferred to media in order to assess ILTV replication. Viral DNA was extracted from these samples using VX Universal Liquid Sample DNA Extraction Kit (Qiagen) and a Corbett X-tractor Gene Robot (Corbett Robotics, Australia), as described above.

Results

Results from the transmission study

Detection of ILTV DNA in conjunctival or tracheal samples

Viral DNA was detected in 15 of the swabs collected from the in-contact birds. A total of 13 in-contact birds were classified as infected by applying the case definition of infection as outlined above. The number of positive samples collected at each site (trachea or conjunctiva) and the number of infected and uninfected birds in each experimental group is summarised in Table 1. The majority of positive samples were collected 4 days after exposure. This was consistent between naïve birds infected with wt ILTV or ΔgG ILTV (75% and 80% of positive swabs, respectively). The only positive sample in the vaccinated in-contact birds was also collected 4 days after exposure. All of the negative DNA extraction control samples were negative for the presence of ILTV DNA. All of the positive DNA extraction control samples were positive for the presence of ILTV DNA.

Table 1. *Summary of the number of ILTV positive samples collected at each site (trachea or conjunctiva) and the number of infected and uninfected birds in each experimental group.*

	ILTV positive samples at each site Number (proportion)		Infected and uninfected birds Number (proportion)	
Experimental group	Trachea	Conjunctiva	Infected	Uninfected
wt ILTV in naïve birds	1 (0.25)	3 (0.75)	4 (0.13)	26 (0.87)
ΔgG ILTV in naïve birds	8 (0.80)	2 (0.20)	8 (0.27)	22 (0.73)
wt ILTV in vaccinated birds	0 (0.00)	1 (1.00)	1 (0.03)	29 (0.97)

Reproductive rates of ILTV infection

To calculate reproductive rates of ILTV infection the number of infected in-contact birds in each replicate of each experimental group was determined. Reproductive rates for each replicate were calculated and used to determine the mean reproductive rate and 95% confidence interval for each experimental group. These results are shown in Table 2.

Table 2. *Reproductive rates of ILTV infection in naïve or vaccinated birds*

	Reproductive rate			
Experimental group	Replicate 1	Replicate 2	Replicate 3	Mean (95% CI)
wt ILTV in naïve birds	0	2	2	1.33 (0.03, 2.64) [A]
ΔgG ILTV in naïve birds	0	4	4	2.67 (0.05, 5.28) [A]
wt ILTV in vaccinated birds	1	0	0	0.33 (0.00, 0.99) [A]

[A] Values with the same superscript lettering were not significantly different (student t test)

The effect of vaccination on ILTV infection

A greater proportion of naïve birds became infected with wt ILTV compared to vaccinated birds (Table 1). The attributable fraction (exposed) (AF_e) was calculated to measure the effect of not vaccinating the in-contact birds on transmission of wt ILTV. The AF_e value was 0.77 thus indicating that the vaccine prevented 77% of the cases of infection (over one transmission cycle) that would have occurred in the vaccinated group if the vaccine had not been used. To measure the association between not vaccinating the in-contact birds and becoming infected with wt ILTV the risk ratio (RR) was

determined. The RR value was 4.00 (95% confidence interval of 0.64 to 26.37). An RR value of 4.00 indicates that infection was 4 times more likely to spread from an infected bird to an unvaccinated bird then from an infected bird to a vaccinated bird.

The association between the site of ILTV detection and strain of ILTV

Viral DNA could be detected in the conjunctiva in a greater proportion of in-contact birds infected with wt ILTV, compared to those infected with ΔgG ILTV (Table 1). The odds ratio (OR) was calculated to determine the association between infection with wt ILTV and detection of ILTV DNA in the conjunctiva. The OR value was 16.00 (95% confidence interval of 1.34 to 171.96). This indicates that there is 16 times the chance of detecting ILTV DNA in the conjunctiva of a bird infected with wt ILTV, compared to a bird infected with ΔgG ILTV. Similarly there is 16 times the chance of detecting ILTV DNA in the trachea of a bird infected with ΔgG ILTV, compared to a bird infected with wt ILTV.

Weight gain

The percentage weight gain of the in-contact birds was assessed as a measure of virus virulence. Figure 1 shows the mean and standard deviation of weight gain in different categories of in-contact birds.

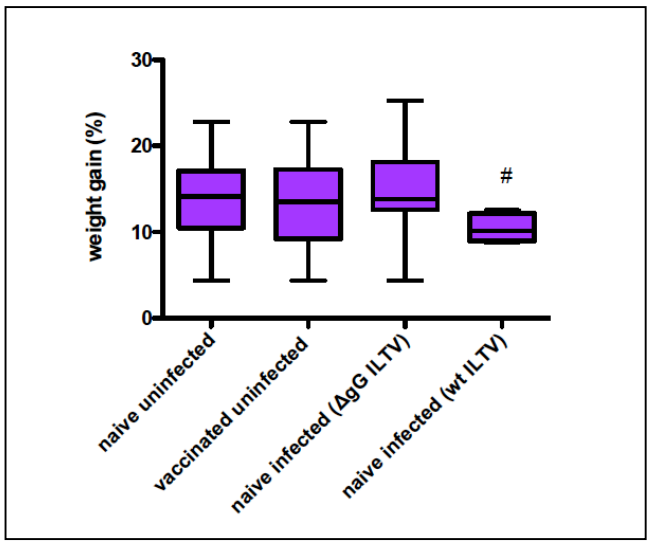

Figure 1. *Percentage weight gain of infected or uninfected in-contact birds. A student t test was used to compare the weight gain in each group to the weight gain in naïve, uninfected birds. A significant difference was observed only in the group of naïve birds infected with wt ILTV (indicated with #). Only one vaccinated, in-contact bird became infected with wt ILTV. This bird had a weight gain of 15.3%.*

Results from the vaccine study

Vaccine safety

Vaccine safety was assessed by comparing clinical signs (Table 3) and weight gain (Figure 2) between the different groups of vaccinated birds and unvaccinated (control) birds. One bird in the group vaccinated with SA2 ILTV died after vaccination. Post mortem analysis revealed tracheal pathology consistent with ILTV-induced disease. Data from this bird was not included in Table 3 or Figure 2.

Table 3. *Clinical scores 5 days after vaccination*

Vaccine strain	Clinical score Demeanour Medium (range)	Clinical score Dyspnoea Medium (range)	Clinical score Conjunctivitis Medium (range)	Clinical score Overall Medium (range)
Unvaccinated	0 (0 – 1) ^{A}	0 (0 – 2) ^{A}	0 (0 – 0) ^{A}	0 (0 – 2) ^{A}
SA2 ILTV	1 (0 – 1) ^{B}	2 (0 – 3) ^{B}	0 (0 – 1) ^{B}	3 (0 – 5) ^{B}
A20 ILTV	1 (0 – 1) ^{B}	1 (0 – 2) ^{C}	0 (0 – 2) $^{A, B}$	2 (0 – 5) ^{C}
Serva ILTV	0 (0 – 1) ^{C}	0 (0 – 2) $^{A, D}$	0 (0 – 1) $^{B, C}$	1 (0 – 4) ^{C}
ΔgG ILTV	0 (0 – 1) $^{A, C}$	1 (0 – 2) $^{C, D}$	0 (0 – 0) ^{A}	1 (0 – 3) ^{C}

$^{A, B, C, D}$ Values with the same superscript lettering in the same column were not significantly different.

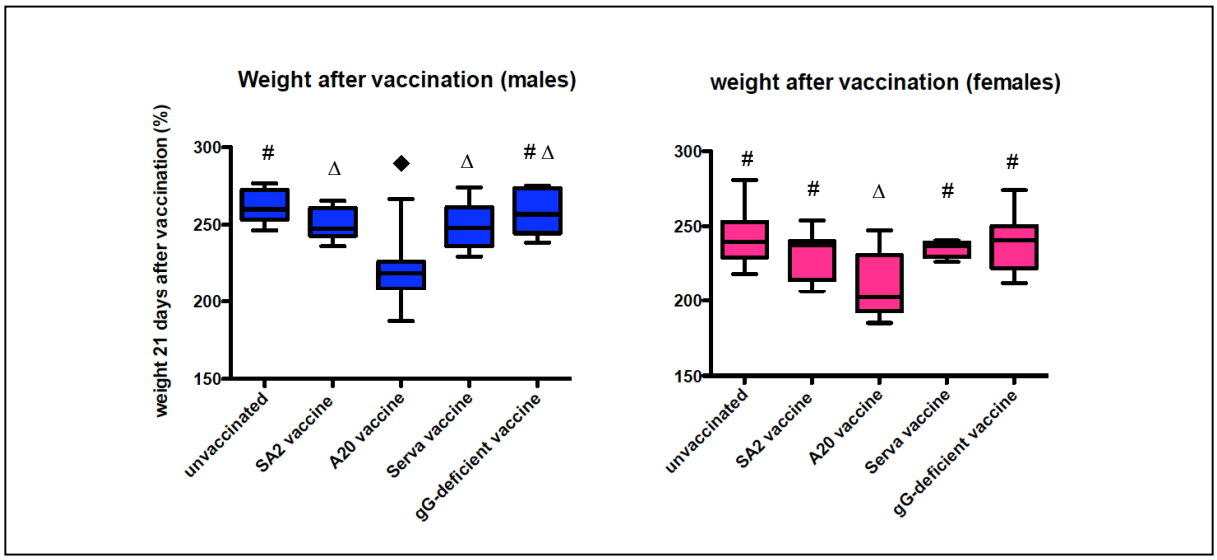

Figure 2*: Bird weight 21 days after vaccination (expressed as a percentage of weight at vaccination). Groups marked with the same symbols (#, Δ,◆) in the same panel were not significantly different.*

Vaccine efficacy

Vaccine efficacy was assessed by comparing clinical signs, tracheal pathology (Table 4) and weight gain (Figure 3) between groups of vaccinated and unvaccinated (control) birds following challenge with virulent virus. One bird in the unvaccinated group died after challenge. Post mortem analysis revealed tracheal pathology consistent with ILTV-induced disease. Data from this bird was included in Table 4 and Figure 3.

Table 4. *Clinical scores 5 days after challenge and tracheal pathology scores 6 days after challenge*

Vaccine strain	Clinical score Demeanour Medium (range)	Clinical score Dyspnoea Medium (range)	Clinical score Conjunctivitis Medium (range)	Clinical score Overall Medium (range)	Tracheal pathology score Medium (range)
Unvaccinated	1 (0 – 2) ^{A}	0 (0 – 4) ^{A}	0 (0 – 1) ^{A}	1 (0 – 7) ^{A}	4 (0 – 5) ^{A}
SA2 ILTV	0 (0 – 1) ^{B}	0 (0 – 2) ^{A}	0 (0 – 0) ^{A}	0 (0 – 2) ^{A}	1 (0 – 2) ^{B}
A20 ILTV	0 (0 – 1) ^{B}	0 (0 – 2) ^{A}	0 (0 – 0) ^{A}	0 (0 – 3) ^{A}	1 (0 – 2) ^{B}
Serva ILTV	1 (0 – 2) ^{A}	0 (0 – 2) ^{A}	0 (0 – 0) ^{A}	1 (0 – 3) ^{A}	1 (0 – 3) ^{B}
ΔgG ILTV	0 (0 – 1) $^{A, B}$	0 (0 – 2) ^{A}	0 (0 – 1) ^{A}	0 (0 – 3) ^{A}	1 (0 – 4) ^{B}

$^{A, B,}$ Values with the same superscript lettering in the same column were not significantly different.

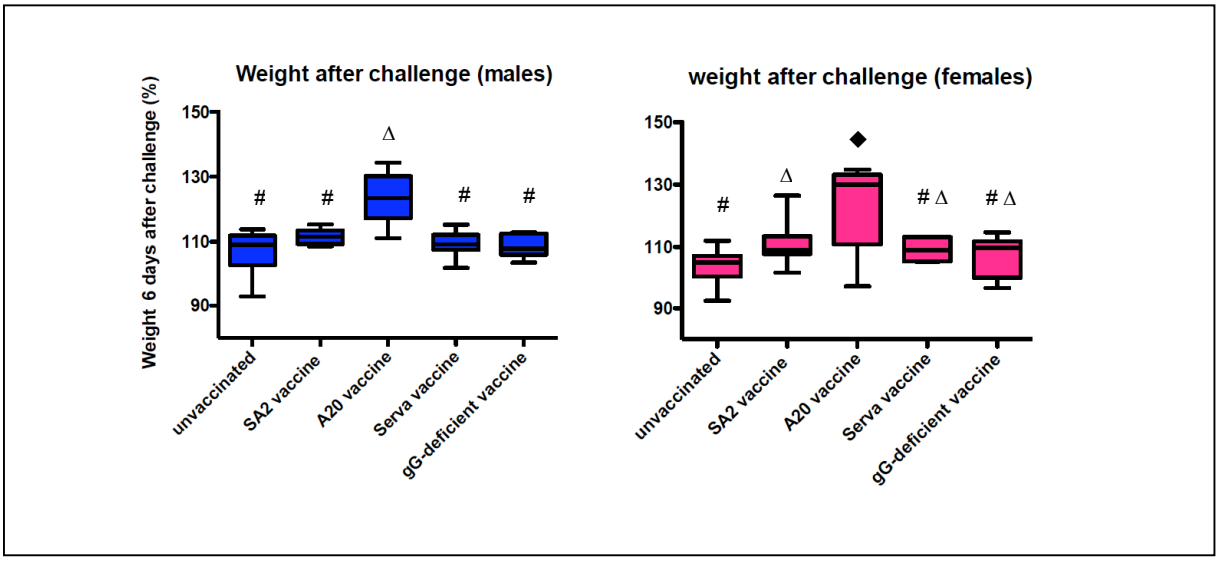

Figure 3*: Bird weight 6 days after challenge (expressed as a percentage of weight at challenge). Groups marked with the same symbols (#, Δ,◆) in the same panel were not significantly different.*

Discussion

This project was successful in achieving its objectives. Some minor modifications to the original experimental design (as outlined in the project proposal) were implemented in order to manage time and equipment constraints and to improve experimental results. This included changing the method of challenge in the vaccine study from an indirect challenge (by contact with infected birds) to a direct intra-tracheal challenge. In addition this project has involved adapting the real-time PCR protocol used to quantify ILTV genome copies to a new, more sensitive machine that uses more cost-effective reagents. Analysis of tracheal viral replication following challenge using this new protocol is ongoing and so this data has not been included in this report. The ability of the different vaccine strains to establish latency in vaccinated birds was not assessed in this project, as was originally indicated, due to time constraints.

The findings from this project support the use of gG-deficient ILTV as an attenuated vaccine. This candidate vaccine was effective at reducing transmission of virulent ILTV from infected to uninfected birds. The effective reproductive rate of virulent ILTV in vaccinated birds was < 1. This indicates that infection with virulent ILTV would be eliminated in a population of birds vaccinated with gG-deficient ILTV. The attenuated phenotype of gG-deficient ILTV (as indicated by analysis of weight gain) was shown to be stable after one *in vivo* passage and subsequent natural infection of in-contact birds. This is a significant potential advantage of this vaccine compared with conventionally attenuated vaccines that can revert to high levels of virulence following *in vivo* passage. In addition the weight gain of the uninfected vaccinated birds was not significantly different to the naïve, uninfected birds. This is another important consideration for vaccine safety.

Direct comparison of this gG-deficient ILTV vaccine with Australian (commercial) ILTV vaccines confirmed its high level of vaccine safety as assessed by analysis of clinical signs and weight gain. The efficacy of the gG-deficient ILTV vaccine was similar to the commercial ILTV vaccines, as assessed by analysis of clinical signs and tracheal pathology following challenge with virulent virus. Birds vaccinated with A20 ILTV showed significantly greater weight gains after challenge than birds vaccinated with all other vaccines, however this should be considered together with the significantly lower weight gains in this group following vaccination. Serum samples collected from vaccinated birds in this experiment have been stored in our laboratory. These samples will be useful for future studies that aim to develop an ELISA to differentiate birds infected with virulent ILTV from birds vaccinated with gG-deficient ILTV.

Implications

This gG-deficient ILTV vaccine has the potential to improve control of ILT in Australian poultry. Benefits of this vaccine, over conventionally attenuated vaccines, include enhanced vaccine safety and the potential to serologically differentiate vaccinated birds from infected birds. Differentiating between infected and vaccinated birds is useful in disease eradication programs. This approach has been used successfully to eradicate diseases at regional and national levels. For example in the USA a program of vaccination using a gG-deficient vaccine strain of porcine herpesvirus 1 (PRV-1) is being successfully combined with the use of a differential ELISA in a national eradication campaign [9].

Infectious laryngotracheitis is currently causing direct and widespread loss to poultry producers in Australia, as well losses to businesses connected with poultry production. Control of ILT is based on biosecurity and vaccination [10]. These control measures are implemented at a significant economic cost to producers. Producers experience stock and production losses caused by vaccination with conventionally attenuated ILT vaccines. These losses can be due to insufficient attenuation of the vaccine strain or reversion to virulence following *in vivo* passage [11]. There is also some concern that these vaccines preparations can include a small amount of other (virulent) strains of ILTV that can cause disease following *in vivo* amplification. This gG-deficient ILTV vaccine has the potential to avoid these problems.

Recommendations

The findings from this project support the continued development of gG-deficient ILTV as an attenuated vaccine for use in Australian and overseas. To take full advantage of the high level of safety of gG-deficient ILTV it is recommended that future studies commence with an investigation into administering higher doses of this vaccine (a dose-response study). This has not been investigated previously. It is likely that a higher level of vaccine efficacy can be achieved by optimising the vaccine dose. It is also recommended that future studies investigate the development of a differential ELISA to discriminate between vaccinated and infected birds (based on the presence or absence of serum antibodies against gG). This would be beneficial in ILT eradication programs.

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Plain English Compendium Summary

Project Title:	
Project No.:	09-06 UoM
Researcher:	Dr Joanne Devlin
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Phone:	Phone: 03 9035 8110
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Objectives	To further characterise a novel recombinant vaccine to control infectious laryngotracheitis
Background	Infectious laryngotracheitis (ILT) is an acute respiratory disease caused by infection with infectious laryngotracheitis virus (ILTV). This alphaherpesvirus is a significant concern for the poultry industry in Australia and overseas. To enhance control of ILT we have previously generated a novel candidate vaccine strain of ILTV that is deficient in the virulence factor glycoprotein G (gG). In this project this gG-deficient ILTV was further characterised in order to assess its potential to improve control of ILT.
Research	This research project was divided into two components. The first component assessed ILTV transmission between birds. The focus of this part of the study was to assess the ability of the vaccine to prevent transmission of virulent virus between birds. This component of the study also examined the transmission characteristics of gG-deficient ILTV compared with a virulent strain of virus. The second component of this study compared the safety and efficacy of this gG-deficient ILTV vaccine to other (commercial) ILTV vaccines that are available in Australia.
Outcomes	Research findings from the transmission study showed that this novel vaccine was effective at reducing transmission of virulent virus between birds. The vaccine prevented 77% of cases of transmission of infection over one transmission cycle. Additional calculations indicated that the degree of transmission of virulent virus in vaccinated birds was sufficiently low as to result in the elimination of virulent virus in populations of birds vaccinated with gG-deficient ILTV. The study also showed that this vaccine strain was able to spread from bird-to-bird. Importantly this vaccine remained safe and attenuated following bird-to-bird spread. Comparison of this vaccine strain with other ILTV vaccine strains showed that gG-deficient ILTV has a high level of vaccine safety and a similar level of vaccine efficacy compared to the commercial ILTV vaccines that are currently used in Australia.
Implications	The findings from this project point to the suitability of gG-deficient ILTV for use as a vaccine to control ILT. This gG-deficient ILTV vaccine has the potential to improve control of ILT in Australian poultry industries. Benefits of this vaccine, over conventionally attenuated vaccines, include enhanced vaccine safety and the potential to serologically differentiate vaccinated birds from infected birds. This feature is useful in disease eradication programs.
Publications	Two manuscripts describing these studies are currently being prepared. These manuscripts will be submitted for publication in international, peer-reviewed journals.