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Developing novel antigen delivery systems; ILTV as a vaccine vector

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Investigations into a novel recombinant vaccine to control infectious laryngotracheitis virus
Project No: 09-06 UoM (Devlin)

Researcher Contact Details

Dr Joanne Devlin
The School of Veterinary Science,
The University of Melbourne
Parkville, VIC, 3010

Phone: 03 9035 8110
Fax: 03 8344 7374
Email: devlinj@unimelb.edu.au

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

Australian Poultry CRC Contact Details

PO Box U242
University of New England
ARMIDALE NSW 2351

Phone: 02 6773 3767
Fax: 02 6773 3050
Email: info@poultrycrc.com.au
Website: http://www.poultrycrc.com.au

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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. Importantly this attenuated gG-deficient is also suitable as a vector for the expression of immunogenic proteins of other chicken pathogens. The objective of this proof-of-concept project was to explore the use of this vaccine strain of ILTV as a vector to express antigens from infectious bronchitis virus (IBV).

The first component of this project aimed to assemble a plasmid containing PCR-amplified DNA from both ILTV and IBV for use in co-transfection experiments to generate recombinant virus. In this project DNA fragments encoding immunogenic regions of the spike (S) and nucleocapsid (N) genes of IBV were generated and assembled together with ILTV DNA sequence extending upstream and downstream form the gG gene. These PCR products were assembled in the order ILTV upstream DNA – IBV S gene DNA – IBV N gene DNA – ILTV downstream DNA using overlap extension PCR. This product was cloned and the sequence was verified.

The second component of this project aimed to generate recombinant virus using homologous recombination. This process involves co-transfecting three separate DNA preparations into the nucleus of LMH cells (a chicken hepatoma cell line) and then selecting recombinant viruses for plaque purification and characterisation. These three different samples of DNA include the plasmid described above, ILTV genomic DNA from an intermediate strain of ILTV that expresses green fluorescent protein (GFP) in place of gG and a plasmid expressing the ICP-4 gene of ILTV. To this end these DNA preparations were generated. The ability of the ILTV genomic DNA to establish infection following transfection was verified. Co-transfection experiments are currently underway to generate recombinant virus.

This project was successful in achieving several objectives. The remaining objective will be completed in 2010. The laboratory work in this project (as described in the original timeline) was originally to be performed by experienced researchers. Subsequent to this, and with the approval from the CRC, a postgraduate student was identified to become involved in this project. Concomitant with the additional time required for postgraduate research training, this project has not progressed according to the original timeline described. Instead the work described in this project is ongoing. Completion is expected in 2010. In the longer term benefits to the poultry industry are expected to arise as a result of this postgraduate involvement.

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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]. 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, 3]. Importantly this attenuated ΔgG ILTV vaccine is also suitable as a vector for the expression of immunogenic proteins of other chicken pathogens.

The large, double-stranded DNA genome of ILTV facilitates the successful application of recombinant DNA techniques. We have shown that ILTV (including ΔgG ILTV) can be utilised to express foreign antigens [4] [2]. This proposed project aimed to generate a proof-of-concept recombinant ΔgG ILTV vaccine that expresses antigen from infectious bronchitis virus (IBV). Infectious bronchitis virus is a coronavirus that causes economically significant disease in chickens. The single-stranded, positive-sense RNA genome of IBV encodes four structural proteins, including the immunogenic spike (S) glycoprotein and nucleocapsid (N) protein.

Objectives

This strategic project aimed to use gG-deficient ILTV to express antigenic proteins from IBV. This constitutes a proof-of-concept project for using ILTV as a vaccine vector. The first component of the project aimed to assemble a plasmid containing PCR-amplified DNA from both ILTV and IBV for use in co-transfection experiments to generate recombinant virus. The second component of this project aimed to generate recombinant virus using co-transfection and homologous recombination.

Methodology

Methodologies utilized in plasmid construction

The first component of this project aimed to assemble a plasmid containing PCR-amplified DNA from both ILTV and IBV for use in co-transfection experiments to generate recombinant virus. Plasmid pILTVgGup-IBS-IBN-ILTVgGd was constructed to contain immunogenic regions of the S and N genes of IBV flanked by sequences that extend 1004 bp upstream (gGu) and 989 bp downstream (gGd) of the gG gene of ILTV, respectively. The DNA from the ILTV gG flanking regions was assembled together with the DNA encoding for regions of the S and N genes of IBV using the technique of gene splicing by overlap extension PCR (SOE PCR) [5]. The resultant construct (ILTVgGup-IBS-IBN-ILTVgGd) was ligated into the plasmid pGEM-T (Promega) to form pILTVgGup-IBS-IBN-ILTVgGd.

ILTVgGu and ILTVgGd were amplified by PCR using an iCycler Thermal Cycler. The 50 μl reaction mixture contained 2 mM $MgSO_4$, 1.25 units of Platinum *Taq* High Fidelity DNA Polymerase (Invitrogen), 1 x Platinum *Taq* High Fidelity DNA Polymerase Buffer (Invitrogen), 200 μM of each dNTP (Promega), 0.2 μM of the primers 1 and 2 or 3 and 4 for the segments Gu and Gd, respectively

(Table 1), and 2 μl of extracted ILTV DNA, or sterile water for the contamination control reactions. The reactions were incubated through 1 cycle of 94ºC for 3 min, followed by 30 cycles of denaturation at 94ºC for 30 s, annealing at 58.3ºC for 30 s and extension at 68ºC for 1 min and then one final extension cycle of 68ºC for 5 min. The PCR to amplify regions of the S and N genes from IBV utilised the same conditions and reactions components, except for primers and template. Amplification of the IBV S gene utilised primers 5 and 6 (Table 1). Amplification of the IBV N gene utilised primers 7 and 8 (Table 1). Plasmid DNA containing the S and N genes of the VicS strain of IBV (constructed previously in our laboratory) was used as template in these reactions. The PCR products from each reaction were separated and visualised by agarose gel electrophoresis using either a 1% or 2% agarose gel. Bands of the expected size were identified by comparison to standard markers and then the DNA product was extracted using the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions.

***Table 1**: Primers used in this study*

Primer	Orientation	Sequence (5' to 3')	Product
1	Forward	gctgggctgtttgtcagagta	ILTVgGu
2	Reverse	AAAAGTAAAATTAGTTAACGTcatgatgtctcttcagactt	
3	Forward	TAGGAGAGAATGAACTTTGAccacccgagagtgtttttg	ILTVgGd
4	Reverse	ccaagaacccgtcagaaac	
5	Forward	aagtctgaagagacatcatgACGTTAACTAATTTTACTTTTTC	IBV S
6	Reverse	TCTGGTTGAAGTTTGGGCGTtgatccattggtgagttta	
7	Forward	taaactcaccaatggatcaACGCCCAAACTTCAACCAGAT	IBV N
8	Reverse	caaaaaacactctcgggtggTCAAAGTTCATTCTCTCCTA	

The four PCR products were assembled together in the order ILTVgGup-IBS-IBN-ILTVgGd using SOE PCR. Three consecutive SOE PCRs were performed. Firstly IBS and IBN were assembled together. The 50 μl PCR mixture contained 2 mM MgSO4, 1.25 units of Platinum *Taq* High Fidelity DNA Polymerase, 1x Platinum *Taq* High Fidelity DNA Polymerase Buffer, 200 μM of each dNTP and 0.2 μM of primers 5 and 8 (Table 1) The template DNA consisted of 2 μl of each of the extracted products of the IBS and IBN PCRs. The reactions were incubated at 94ºC for 2 min, followed by 4 cycles of denaturation at 94ºC for 30 s, annealing at 64ºC for 1.5 min and extension at 68ºC for 3 min, then 26 cycles of denaturation at 94ºC for 30 s, annealing at 64ºC for 45 s and extension at 68ºC for 3 min, with one final extension cycle of 68ºC for 5 min. The SOE PCR products were separated and visualised by agarose gel electrophoresis and extracted using the QIAquick Gel Extraction Kit. Secondly ILTVgGd was added to the IBS-IBN DNA fragment. This SOE PCR utilised the same conditions and reaction components except that the primers used were primers 4 and 5 (Table 1) and the template DNA consisted of 2 μl of each of the extracted products of the IBS-IBN and ILTVgGd PCRs. The SOE PCR products were separated and visualised by agarose gel electrophoresis and extracted using the QIAquick Gel Extraction Kit. Finally ILTVgGu was added to the IBS-IBN-ILTVgGd DNA fragment. This SOE PCR utilised the same conditions and reaction components except the primers used were primers 1 and 4 (Table 1) and the template DNA consisted of 2 μl of each of the extracted products of the IBS-IBN-ILTVgGd and ILTVgGu PCRs. The PCR products from this final SOE PCR were separated and visualised by agarose gel electrophoresis and extracted using the QIAquick Gel Extraction Kit.

Following extraction, the PCR product ILTVgGup-IBS-IBN-ILTVgGd was ligated into the plasmid pGEM-T using the pGEM-T Vector System 1 kit (Promega) according to the manufacturer's instructions. Ligation products were used to transform *E. coli* DH5α (Invitrogen) by electroporation. Transformants were selected by blue-white screening on LB plates containing ampicillin, IPTG and X-gal after incubation overnight at 37ºC. White colonies (indicating an insertion event) were picked

and grown overnight in LB broth containing 100 μg ampicillin /ml. Plasmids were extracted using the Flexiprep extraction kit (Amersham Biosciences) according to the manufacturer's instructions. Gel electrophoresis was used to identify plasmids of the anticipated size and the insertion product was sequenced using the primers in Table 1 and Big Dye Terminator (BDT) version 3.1 chemistry (ABI PRISM) according to manufacture's instructions. Sequencing reactions were incubated in an iCycler Thermal Cycler (Biorad). Sequence chromatograms were examined using GENEius (BioLink). Sequence alignments were performed using ClustalW [6]. Plasmid DNA for use in co-transfections was then prepared from overnight cultures of transformed *E. coli* grown in LB broth containing 100 μg ampicillin/ml, using the Midiprep plasmid extraction kit (Qiagen) according to the manufacturer's instructions.

Methodologies utilized in co-transfection experiments

The second component of this project aimed to generate recombinant virus using homologous recombination. This process involves co-transfecting three separate DNA preparations into the nucleus of LMH cells (a chicken hepatoma cell line) and then selecting recombinant viruses for plaque purification and characterisation. These three different samples of DNA include the plasmid described above, ILTV genomic DNA from an intermediate strain of ILTV that expresses green fluorescent protein (GFP) in place of gG (ΔgG-GFP-ILTV) and a plasmid expressing the ICP-4 gene of ILTV.

The expression plasmid pRc-ICP4 was kindly provided by Prof T. C. Mettenleiter (Federal Research Centre for Virus Diseases of Animals, Insel Riems, Germany). This plasmid was constructed by the insertion of the ICP4 gene of ILTV into pRc-CMV (Invitrogen) [7]. The plasmid was used to transform *E. coli* DH5α by electroporation and transformants selected after overnight incubation on agar plates containing 100 μg ampicillin/ml. Plasmid DNA for co-transfections was prepared from overnight cultures of transformed *E. coli* containing pRc-ICP4 in LB containing 100 μg ampicllin/ml using the Midi-prep plasmid extraction kit.

ILTV DNA for use in co-transfection experiments was extracted from fluid collected from the allantoic cavity of specific-pathogen free (SPF) eggs inoculated with ΔgG-GFP-ILTV. Thirty SPF eggs, at 10 days of age, were inoculated with 100 μl of virus stock material into the allantoic sac. The eggs were incubated at 37°C in a humidified environment for 7 days. The allantoic fluid was collected and clarified. Virions were pelleted by centrifugation at 50,000 g for 1 hour in a Beckman Ultrafuge. Pelleted virions were re-suspended in 20 mM tris(hydroxymethyl)aminomethane (Tris)-HCl (pH 7.4), 1 mM ethylenediaminetetraacetic acid (EDTA) and 150 mN NaCl and then purified using a 5 – 15% Ficoll gradient. Viral DNA was extracted from purified virions using the High Pure PCR Product Purification Kit (Roche). The concentration of DNA was determined by spectrophotometry. To determine the infectivity of the prepared ILTV DNA, approximately 0.25 μg of ILTV DNA and 1 μg of pRc-ICP4 was used to co-transfect sub-confluent LMH cells, grown in 6 well plates, using FuGene 6 reagent (Roche) according to the manufacturer's instructions. A 4:1 ratio of reagent:DNA provided maximum transfection efficiency. The medium containing Fugene 6 was removed from the cells 12 hours after application, because of its cytotoxic effects, and replaced with standard media. Incubation was then continued until viral plaques became visible. These LMH cells are a chicken hepatoma cell line [8]. Cells were cultured as previously described [4].

Currently, co-transfection experiments are underway to generate recombinant virus. To this end ILTV DNA, pRc-ICP4 and pILTVgGup-IBS-IBN-ILTVgGd have been co-transfected into LMH cells. Ongoing studies will screen the resulting viruses for expression of GFP. Viral plaques that do not express the GFP marker will be selected and plaque purified. The recombination region of the ILTV genome of the plaque-purified virus will be sequenced to confirm successful integration of the IBV S and N genes into the genome of ΔgG ILTV.

Results

Plasmid construction

Sequence analyses confirmed the integrity of the assembled DNA inserted into pILTVgGup-IBS-IBN-ILTVgGd. Appendix 1 shows the sequence of the cloned insert (ConSeq) compared with the expected sequence (RefSeq).

Co-transfection experiments

Transfection of ΔgG-GFP-ILTV DNA into LMH cells showed that the viral DNA was able to establish infection (Figure 1). Co-transfection studies with pILTVgGup-IBS-IBN-ILTVgGd, pRc-ICP4 and ILTV DNA are currently underway to generate recombinant virus.

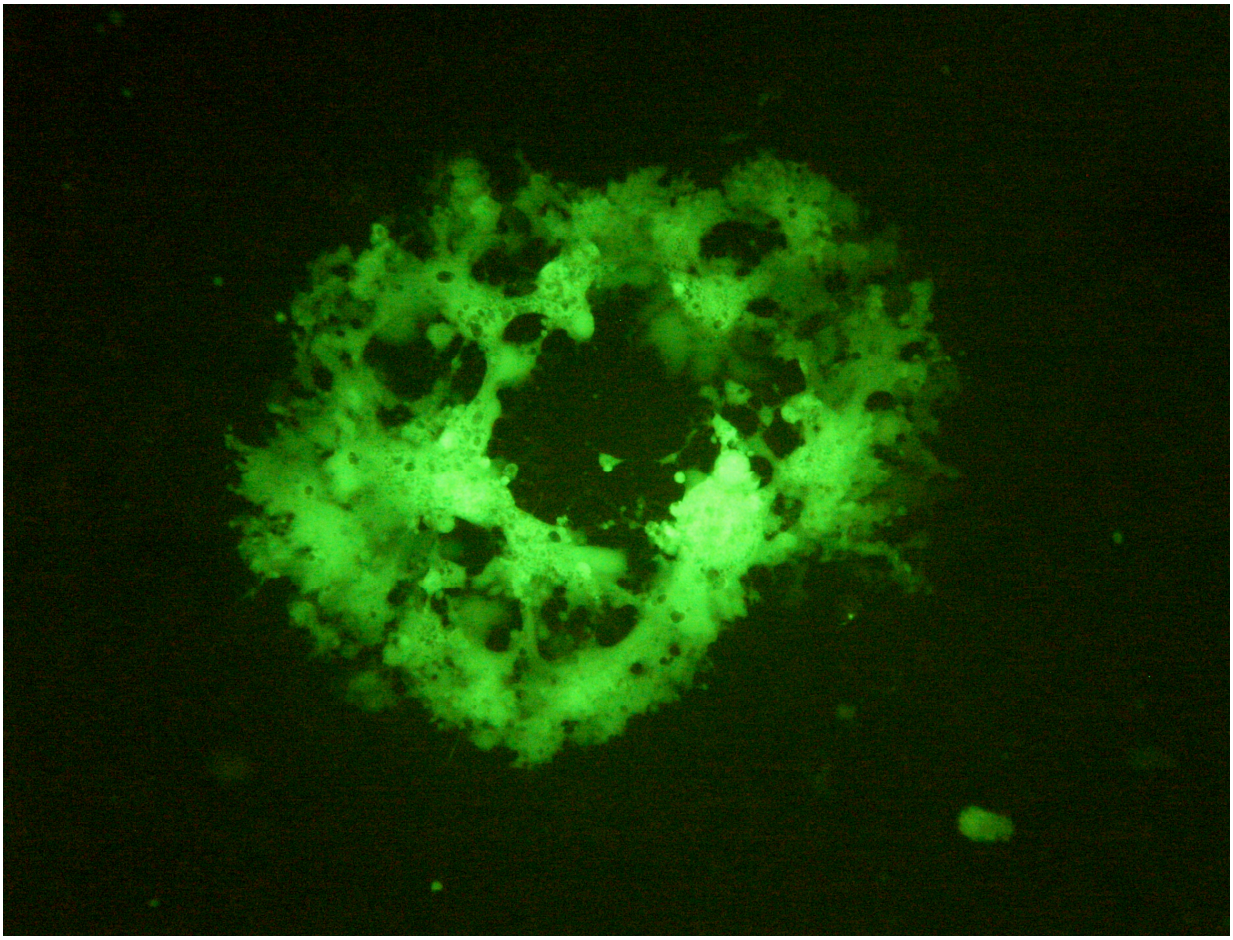

***Figure 1**: Photomicrograph taken using fluorescence microscopy showing a viral plaque in LMH cells transfected with ΔgG-GFP-ILTV DNA.*

Discussion

This project was successful in achieving several objectives. The first component of the project was successfully completed. This resulted in the construction of pILTVgGup-IBS-IBN-ILTVgGd. The second component of the project progressed successfully to the point where all three DNA preparations required to generate recombinant virus were constructed. This included the preparation of viral DNA from ΔgG-GFP-ILTV. The method used in this study to purify the viral DNA for successful transfection is an improved method compared to our previous work [4] and is expected to assist the construction of recombinant viruses in this project and future projects.This viral DNA was able to establish infection in LMH cells following transfection. Co-transfection experiments utilising all three DNA preparations are currently underway to generate recombinant virus.

The laboratory work in this project (as described in the strategic project protocol form) was originally to be performed by experienced investigators. Subsequent to this, and with the approval from the CRC, a postgraduate student was identified to become involved in this project (Mr Niraj Shil, The University of Melbourne). Concomitant with the additional time required for postgraduate research training, this project has not progressed according to the original timeline described. Instead the work described in this project is ongoing. Completion is expected in 2010.

Implications

This project investigated the feasibility of using gG-deficient ILTV as a vaccine vector. This vector has the potential to be used to deliver antigens from a range of poultry pathogens. The implications of this project will be better able to be assessed when the final objective has been completed. The new Poultry CRC will be able to assess the implications of this project when future research proposals (following on from this project) are submitted to the CRC for funding consideration.

The laboratory work described in this project was necessarily delayed due to additional time required for postgraduate research training. Although this delayed the timeline of this project, the project is ongoing and in the longer term benefits to the poultry industry are expected to arise as a result of engaging young scientists in the field of avian health research.

Recommendations

The studies described in this project progressed successfully but the timeline of the project was delayed. It is recommended that the final component this project be completed as outlined in the project proposal.

Acknowledgements

The investigators thank Dr Philip Markham for contributing his advice and expertise. The investigators gratefully acknowledge the involvement of Niraj Shil in this project and also thank Nino Ficorilli, Paola Vaz and Cynthia Brown for their excellent assistance.

Appendix 1: sequence analysis

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CLUSTAL 2.0.12 multiple sequence alignment

                ILTVgGu
                ------>
ConSeq          GCTGGGCTGTTTGTCAGAGTACTTTGCACTAGTGGTATTACTGGCCGAGACGGTCTTAGC 60
RefSeq          GCTGGGCTGTTTGTCAGAGTACTTTGCACTAGTGGTATTACTGGCCGAGACGGTCTTAGC 60
                ************************************************************

ConSeq          GACCATGTTCGACCACGTACTGGTATTCATGAGGGCGCTGGCAGACGGCAATTTCGATGA 120
RefSeq          GACCATGTTCGACCACGTACTGGTATTCATGAGGGCGCTGGCAGACGGCAATTTCGATGA 120
                ************************************************************

ConSeq          CTATGACGAAACTAGATATATAGACCCCGTTAAAAACGAGTACCTGAACGGAGCCGAGGG 180
RefSeq          CTATGACGAAACTAGATATATAGACCCCGTTAAAAACGAGTACCTGAACGGAGCCGAGGG 180
                ************************************************************

ConSeq          GACTCTGTTACGAGGCATAATGGCCTCCAACACCGCTCTGGCGGTGGTTTGCGCAAACAC 240
RefSeq          GACTCTGTTACGAGGCATAATGGCCTCCAACACCGCTCTGGCGGTGGTTTGCGCAAACAC 240
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ConSeq          CTATTCGACGATAAGAAAACTCCCGTCCGTGGCAACTAGCGCGTGCAATGTTGCCTACAG 300
RefSeq          CTATTCGACGATAAGAAAACTCCCGTCCGTGGCAACTAGCGCGTGCAATGTTGCCTACAG 300
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ConSeq          GACCGAAACGCTGAAAGCGAGGCGCCCTGGCATGAGCGACATATACCGGATATTACAAAA 360
RefSeq          GACCGAAACGCTGAAAGCGAGGCGCCCTGGCATGAGCGACATATACCGGATATTACAAAA 360
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ConSeq          AGAGTTTTTCTTTTACATTGCGTGGCTCCAGAGGGTTGCAACACACGCAAATTTCTGTTT 420
RefSeq          AGAGTTTTTCTTTTACATTGCGTGGCTCCAGAGGGTTGCAACACACGCAAATTTCTGTTT 420
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ConSeq          AAACATTCTGAAGAGAAGCGTGGATACGGGGGCCCCGCCATTTTTGTTCAGGGCCAGCTC 480
RefSeq          AAACATTCTGAAGAGAAGCGTGGATACGGGGGCCCCGCCATTTTTGTTCAGGGCCAGCTC 480
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ConSeq          GGAGAAGCGGCTGCAGCAGTTAAATAAAATGCTCTGCCCCCTTCTCGTGCCGATTCAATA 540
RefSeq          GGAGAAGCGGCTGCAGCAGTTAAATAAAATGCTCTGCCCCCTTCTCGTGCCGATTCAATA 540
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ConSeq          TGAAGACTTTTCGAAGGCCCTGGGGTCTGAGCTCAAGAGGGAAAAGTTAGAGACATTCGT 600
RefSeq          TGAAGACTTTTCGAAGGCCCTGGGGTCTGAGCTCAAGAGGGAAAAGTTAGAGACATTCGT 600
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ConSeq          TAAAGCTATTTCCAGCGACAGGGACCCGAGGGGGTCCCTAAGATTTCTCATTTCGGACCA 660
RefSeq          TAAAGCTATTTCCAGCGACAGGGACCCGAGGGGGTCCCTAAGATTTCTCATTTCGGACCA 660
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ConSeq          TGCAAGGGAAATTATTGCAGACGGAGTACGGTTTAAGCCGGTGATAGACGAGCCGGTTCG 720
RefSeq          TGCAAGGGAAATTATTGCAGACGGAGTACGGTTTAAGCCGGTGATAGACGAGCCGGTTCG 720
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ConSeq          GGCTTCAGTTGCGCTGAGTACCGCTGCCGCTGGGAAAGTGAAAGCGCGACGCTTAACCTC 780
RefSeq          GGCTTCAGTTGCGCTGAGTACCGCTGCCGCTGGGAAAGTGAAAGCGCGACGCTTAACCTC 780
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ConSeq          AGTTCGCGCGCCCGTACCGGGCGCAGGCGCCGTTTCCGCGCGCCGGAAATCGGAAATATG 840
RefSeq          AGTTCGCGCGCCCGTACCGGGCGCAGGCGCCGTTTCCGCGCGCCGGAAATCGGAAATATG 840
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ConSeq          ATAAAAATGCTTGGCATTTGCGGGCGAAGAGGCGTGATCTGAAGGGCTCCATAATGACGT 900
RefSeq          ATAAAAATGCTTGGCATTTGCGGGCGAAGAGGCGTGATCTGAAGGGCTCCATAATGACGT 900
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ConSeq          AACTGAGCTACGCATCCCTATAAAGTGTACCCGCTGACCGCTAGCCCATACGGTGTTACA 960
RefSeq          AACTGAGCTACGCATCCCTATAAAGTGTACCCGCTGACCGCTAGCCCATACGGTGTTACA 960
                ************************************************************
                                                    ILTVgGu  IBV S
ConSeq          GGAGGGGAGAGAGACAACTTCAGCTCGAAGTCTGAAGAGACATCATGACGTTAACTAATT 1020
RefSeq          GGAGGGGAGAGAGACAACTTCAGCTCGAAGTCTGAAGAGACATCATGACGTTAACTAATT 1020
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ConSeq          TTACTTTTTCAAATGAAAGTAGTGCGCCACCCAATTCTGGTGGTGTAAACACTATTCAAT 1080
RefSeq          TTACTTTTTCAAATGAAAGTAGTGCGCCACCCAATTCTGGTGGTGTAAACACTATTCAAT 1080
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RefSeq          TGTACCAAACTAAAACAGCTCAGAGTGGTTATTATAATTTTAATTTTTCATTTCTGAGTG 1140
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ConSeq          GTTTTGAGTATAAGGAGTTTAATTTTATGTATGGGTCTTATCACCCGCAATGTAACTTTA 1200
RefSeq          GTTTTGAGTATAAGGAGTCTAATTTTATGTATGGATCTTATCACCCGCAATGTAACTTTA 1200
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ConSeq          GACCGGAGACTATTAATAATGGCCTCTGGTTTAACCCACTTTCAGTTTCAATTGCTTACG 1260
RefSeq          TACCGGAGACTATTAATAAAGGCCTCTGGTTTAACTCACTTTCAGTTTCAATTGCTTACG 1260
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ConSeq          GTCCTATTCAAGGCGGTTGCAAGCAATCTGTTTTTAGTGGTAAAGCAACTTGTTGTTATG 1320
RefSeq          GTCCTATTCAAGACGGTTGCAAGCAATCTGTTTTTAGTGGTAAAGCAACTTGTTGTTATG 1320
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ConSeq          CTTACTCATATCGTGGTCCCACAGCGTGTAAAGGTGTTTACAGTGGTGAGTTAACTCAGA 1380
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ConSeq          ATTTTGAATGTGGACTGTTAGTTTTTGTCACTAAGAGCGATGGCTCTCGTATACAAACAG 1440
RefSeq          ATTTTGAATGTGGACTGTTAGTTTTTGTCACTAAGAGCGATGGCTCTCGTATACAAACAG 1440
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ConSeq          CTACCACACCACCAATCATAACTCAACACAATTATAATAATATTACTTTAAATAGTTGTG 1500
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ConSeq          TTGATTATAATATATATGGTAGAACAGGCCAAGGTCTTATTACTAACATAACCGATTCAG 1560
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ConSeq          CTGCTAGTTATAATTATCTAGCAGACGGAGGTTTGGCTATTTTAGATTCATCTGGTGCCG 1620
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IBV S
IBV N
IBV N
ILTVgGd
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RefSeq GGACACTCCTACTTTGCGCACTCGCTACGGTGCTACGGAGGACGAACTTGCACTGGAGCG 2640

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ConSeq          CGGGACGTCCGCCTCAGACGCGGACAACGTGACATTTTCGCTGTCATATCGCCCGCGCCC 2700
RefSeq          CGGGACGTCCGCCTCAGACGCGGACAACGTGACATTTTCGCTGTCATATCGCCCGCGCCC 2700
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ConSeq          AGAAATTCACGGAGCATACTTCACCATAGGGGTATTCGCTACTGGCCAGAGCACGGAAAG 2760
RefSeq          AGAAATTCACGGAGCATACTTCACCATAGGGGTATTCGCTACTGGCCAGAGCACGGAAAG 2760
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ConSeq          CAGCTATTCGGTCATCAGTCGGGTCTTAGTTAACGCCTCTCTGGAACGGTCCGTGCGCCT 2820
RefSeq          CAGCTATTCGGTCATCAGTCGGGTCTTAGTTAACGCCTCTCTGGAACGGTCCGTGCGCCT 2820
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ConSeq          GGAAACGCCGTGCGATGAAAATTTTTTGCAGAACGAGCCTACACGGGACTCGAAGCGTTG 2880
RefSeq          GGAAACGCCGTGCGATGAAAATTTTTTGCAGAACGAGCCTACACGGGACTCGAAGCGTTG 2880
                ************************************************************

ConSeq          GTTAGGCCCCCCGTCGCCTTATGTGCGAGATAACGATGTCGCCGTGTTGACAAAAGCGCA 2940
RefSeq          GTTAGGCCCCCCGTCGCCTTATGTGCGAGATAACGATGTCGCCGTGTTGACAAAAGCGCA 2940
                ************************************************************

ConSeq          GTACATTGGGGAGTGCTACTCCAACTCGGCGGCCCAGACGGGGCTCACGTCTCTCAACAT 3000
RefSeq          GTACATTGGGGAGTGCTACTCCAACTCGGCGGCCCAGACGGGGCTCACGTCTCTCAACAT 3000
                ************************************************************

ConSeq          GACCTTTTTCTATTCGCCTAAAAGAATAGTAAACGTCACGTGGACAACCGGCGGCCCCTC 3060
RefSeq          GACCTTTTTCTATTCGCCTAAAAGAATAGTAAACGTCACGTGGACAACCGGCGGCCCCTC 3060
                ************************************************************

ConSeq          CCCCTCGCGCATAACGGTATACTCGTCGCGGGAGAACGGGCAGCCCGTGTTGAGGAACGT 3120
RefSeq          CCCCTCGCGCATAACGGTATACTCGTCGCGGGAGAACGGGCAGCCCGTGTTGAGGAACGT 3120
                ************************************************************
                      ILTVgGd
                    <-------
ConSeq          TTCTGACGGGTTCTTGG 3137
RefSeq          TTCTGACGGGTTCTTGG 3137
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Plain English Compendium Summary

Project Title:	
Project No.:	09-28 UoM
Researcher:	Dr Joanne Devlin
Organisation:	The University of Melbourne
Phone:	Phone: 03 9035 8110
Fax:	Fax: 03 8344 7374
Email:	devlinj@unimelb.edu.au
Objectives	This 'proof-of-concept' project aimed to explore the use of a novel vaccine strain of infectious laryngotracheitis virus as a vaccine vector
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). Importantly this attenuated gG-deficient ILTV vaccine is also suitable as a vector for the expression of immunogenic proteins from other chicken pathogens.
Research	This *in vitro* research project focused on using gG-deficient ILTV to express antigenic proteins from infectious bronchitis virus (IBV). The IBV antigenic proteins studied were the spike (S) protein and the nucleocapsid (N) protein. The project had two main components. The first component involved generating a DNA construct (a plasmid) containing the relevant regions of ILTV and IBV DNA. The second component of this project aimed to generate recombinant virus using this plasmid in conjunction with two other DNA preparations (another plasmid that expresses an ILTV protein and ILTV genomic DNA).
Outcomes	The first component of the project was successfully completed. Studies in the second component of the project were completed to the point where all three DNA preparations required to generate recombinant virus were generated. The final stages of the work described in this project are ongoing. Completion is expected in 2010.
Implications	The timeline of this project was necessarily delayed due to additional time required for postgraduate research training. In the longer term benefits to the poultry industry are expected to arise as a result of this postgraduate involvement. When completed the work in this project is expected to demonstrate the feasibility of using gG-deficient ILTV as a vaccine vector. This vector has the potential to be used to deliver antigens from a range of poultry pathogens.
Publications	No publications