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Detection of vvIBDV strains and Australian variants in poultry

A report for the Rural Industries Research and Development Corporation

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Foreword

The aims of this project were to: (a) develop diagnostic tests to detect exposure of poultry to very virulent infectious bursal disease virus (IBDV); (b) confirm that all very virulent strains can be differentiated by the methods developed; (c) further characterise already isolated Australian variants and (d) determine the prevalence of variant like strains on other commercial poultry sites in Australia.

Strains similar to vvIBDV strains have not been detected in Australia. As the incursion of such strains would represent a serious threat to the local industry in terms of losses it would be prudent to ensure the existence of adequate techniques and strategies to detect such exposure in the fastest possible way. The possibility that vvIBDV strains could emerge in Australia by mutation of endemic strains should also be considered. The data obtained on the variation occurring in local IBDV strains would further aid in understanding whether significant alterations in antigenicity and pathotype are likely to occur.

This publication summarises results obtained from the 1 July 1997 to 30 June 2000 at the CSIRO Division of Animal Health, Australian Animal Health Laboratory, Geelong. In addition to the principal investigators, Dr S. Sapats, Dr J. Ignjatovic and Mrs. G. Gould, other persons that contributed to the project are Dr H. Heine, Dr S. Prowse , Mr P. Selleck and Ms S. Spiess. Drs A. Gould, H. Westbury, P. Daniels, D. Boyle and Mr T. Pye and Ms J. Kattenbelt were involved in diagnosis of outbreak in NSW in 1999.

This project was funded in part from the Chicken Meat and Egg industry revenues which is matched by funds provided by the Federal Government. In addition to the funds received from RIRDC, CSIRO Division of Animal Health also provided financial, personnel and laboratory support for the project. AusAid also provided funding during 1999-2000.

This report, a new addition to RIRDC's diverse range of over 700 research publications, forms part of our Chicken Meat and Egg R&D program, which aim to support increased sustainability and profitability in the chicken meat industry and to support improved efficiency, sustainability, product quality, education and technology transfer in the egg industry.

Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/reports/Index.htm
- purchases at www.rirdc.gov.au/eshop

Peter Core
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Abbreviations

AAHL	Australian Animal Health Laboratory
AQIS	Australian Quarantine and Inspection Services
Crab	Chicken recombinant antibody
ELISA	Enzyme-linked immunosorbent assay
IBDV	Infectious bursal disease virus
NSW	New South Wales
RNA	Ribonucleic acid
SA	South Australia
VP2	Protective antigen of IBDV
VvIBDV	Very virulent infectious bursal disease virus
WA	Western Australia

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Executive Summary

Objectives of the study

Develop diagnostic tests to detect exposure of poultry to very virulent infectious bursal disease virus (IBDV).

Determine the nucleotide sequence of very virulent IBDV strains from neighbouring countries in order to confirm that all very virulent strains can be differentiated by the methods developed.

Determine the prevalence of variant IBDV strains on commercial poultry sites in Australia.

Further characterise the antigenic and protective capacity of Australian variant IBDV strains.

Background

VvIBDV strains emerged in Europe in 1987 and since then have been detected in many countries including the Middle East, Asia, Africa, and more recently in South America. Australia, New Zealand and the USA are the only countries that remain free from this devastating disease that causes high mortalities and is difficult to control. The potential for transmission of vvIBDV from other countries into Australia is high and, as in other countries, would result in crippling losses. The threat is exacerbated by the highly stable and infectious nature of the virus and more freedom in international trade. The probability that vvIBDV strains can emerge in Australia by mutation of endemic strains should also be considered. For these reasons it would be prudent to ensure that adequate techniques and strategies exist to detect such exposure in the fastest possible way. The current strategy for IBDV strain characterisation is based on nucleotide sequencing, followed by pathogenicity testing and protection studies. The intention in this study was to develop a competitive ELISA in which sera from an already immune flock could be tested in a blocking ELISA to detect exposure to an exotic vvIBDV strains. This was to be achieved by developing a reagent (chicken recombinant antibody) specific for vvIBDV which would then be used in a competitive ELISA.

Initially when this project was conceived only one vvIBDV strain from Asia (OKYM strain from Japan) was characterised although it has been reported that vvIBDV are prevalent in almost all countries throughout Asia. As the presence of vvIBDV strains in neighbouring countries represents the most likely source of incursion of vvIBDV strains into Australia, we aimed to obtain IBDV strains from neighbouring countries and determine their nucleotide sequence. Such sequences would then be compared to sequences of other vvIBDV, as well Australian IBDV strains. This would strengthen our confidence of being able to discriminate an incursion of any vvIBDV, regardless of its origin. We, along with others, have shown previously that nucleotide sequencing of a region termed the hyper variable region within the VP2 gene of IBDV is the only available method that can differentiate vvIBDV strains. This method is of particular importance for Australia as it enables our strains to be clearly differentiated from vvIBDV strains.

In Australia clinical IBD has been uncommon, but in recent times clinically more severe IBD has been observed in some broiler flocks. Although only two strains, 002-73 and V877,

have been available since the disease was first described in 1974, prevailing field strains were considered to be of low pathogenicity. During 1995-1997 RIRDC funded project on IBDV, we showed that in addition to classical strains, variant strains are also present in Australia. Variant strains were isolated from 4 different farms in the state of Victoria, whereas two classical strains were isolated from NSW. Antigenic variation was identified in these strains by using both monoclonal antibodies (in ELISA), and polyclonal chick sera (in virus neutralisation test). Nucleotide sequencing confirmed that strains from Victoria and NSW differ and that they belong to two separate genetic groups, both distant from all overseas strains. In this study we proposed to characterise an additional number of field IBDV isolates that originated from all major poultry producing area in order to determine the distribution of classical and variant types of IBDV across Australia. Additionally, such information would be used to confirm that all Australian strains can be differentiated form overseas IBDV strains at the genetic level, regardless of the site of collection.

During RIRDC 1995-1997 project on IBDV it was also shown that progeny chicks from hens vaccinated with classical vaccines were not protected against challenge with variant strains. Broiler chicks with maternal antibody titres of below 2,000 were susceptible to challenge with variants. In the USA, where antigenic variants have also been detected, bivalent vaccines that include classical and variant strains have been used for vaccination of breeder hens. Also, it has been suggested that vaccines based on the USA variant strains are superior to those based on classical strains. We proposed to compare the protective capacity of inactivated vaccines based on local variant and classical strains, and assess if there is an advantage of variant vaccines in protecting chicks against virulent challenge. Further, although it has been shown by us that the USA and Australian variants differ both antigenically and genetically, it was of interest to assess if inactivated vaccines based on the USA variants are able to provide protection against a challenge with the Australian variants.

Research

(1) Diagnostic tests to detect exposure of poultry to vvIBDV

The intention was to develop a competitive ELISA in which sera from an already immune flock could be tested in a blocking ELISA to detect exposure to exotic vvIBDV strains. This was to be achieved by developing a reagent specific for vvIBDV which would then be used in a competitive ELISA. For the development of a competitive ELISA, chicken recombinant antibody technology was proposed as an approach that could generate reagents specific for vvIBDV strains. Generation of reagents specific for vvIBDV strains using a mouse model has been attempted in the past by other laboratories, without success. The chicken has an advantage since it is the natural host for IBDV and is therefore more likely to see an epitope specific for vvIBDV than a mouse. Initially, we tested the methodology and determined the feasibility of such an approach by attempting to generate chicken recombinant antibody (Crab) specific for the local strain 002/73. We obtained a small panel of Crabs that showed relatively high specificity in ELISA. Generation of these Crabs therefore indicated to us that the approach using chicken recombinant antibody technology was feasible for the generation of antibodies specific for vvIBDV.

Using the same methodology, mRNA was isolated from chicks immunised in Belgium against a vvIBDV and an attempt was made to generate a Crab specific for vvIBDV. At the time we did not have access to vvIBDV in Australia and thus needed to rely on the assistance and importation of reagents from an overseas laboratory. However, we were not able to obtain any specific Crab in this attempt. Following approval from the poultry industry and AQIS, the vvIBDV strain CS88 was imported into AAHL and the generation of an immune library against CS88 was attempted without success. This indicated to us that the original approach used to generate Crabs was of low efficiency and that there were steps limiting the generation of functional antibodies. For this reason an alternative cloning strategy was devised which resulted in the production of a large library against CS88. From this library one clone designated Crab 88 was selected that showed specificity for vvIBDV strain CS88. Crab 88 did not bind to any Australian IBDV strain nor did it react with two European classical strains 52/70 and 1/68. The specificity of Crab 88 needs to be confirmed using a range of vvIBDV strains from different countries as well as a range of classical, variant and vaccine strains.

When Crab 88 was used in a competition ELISA, preliminary data indicated that it was able to block binding of vvIBDV antisera, whereas an IBDV negative sera did not compete for binding of Crab 88 to CS88 antigen. However, antisera directed against other IBDV strains were also able to compete with Crab 88 for binding to CS88 antigen. Further attempts will be made with Crab 88, and other Crabs that are specific for overseas strains, to examine if conditions can be optimised to achieve competition by Crab 88.

(2) Nucleotide sequences of vvIBDV from neighbouring countries

We attempted to obtain IBDV strains from several countries in the region, however, we only received samples from Indonesia. Nucleotide sequencing of samples collected between 1992 and 1995 from broilers and layers suspected to be caused by vvIBDV, confirmed that the majority of strains prevalent in Indonesia were vvIBDV. The majority of Indonesian vvIBDV were identical to the UK661 strain, the prototype of a vvIBDV. Some Indonesian

vvIBDV had one or two unique amino acid changes, however they were still clearly distinguishable as being vvIBDV. These results, together with recently published nucleotide sequences on vvIBDV isolates from France, Germany, Israel, China and Africa, indicated that all vvIBDV strains are genetically almost identical allowing their unambiguous differentiation from all other strains. This finding has consequently strengthened our confidence of being able to discriminate (by nucleotide sequencing) an incursion of any vvIBDV strain into Australia, regardless of its country of origin.

(3) Prevalence of variant IBDV strains on commercial poultry sites in Australia

Ninety one samples were obtained from 20 broiler farms in Victoria (Vic), New south Wales (NSW), Qeensland (Qld), South Australia (SA) and Western Australia (WA). Fifty eight samples from 14 farms were positive for IBDV by an antigen ELISA using Mab 9-6. Of these, forty two samples from 10 broiler farms in NSW (7 farms), Qld (1 farm) and WA (2 farms) were all antigenically identical to vaccine strains using Mabs 17-82, 39A and 9-6. Sequencing of these strains also confirmed that these strains were classical like strains and genetically similar to vaccine strains. They all had similar amino acid sequences to vaccine strains differing by only a few amino acids. In contrast, 16 samples from 4 farms in Victoria and South Australia were all antigenic variants and did not react with Mabs 39A and 44-18. Nucleotide sequencing confirmed that strains from Victoria and SA were antigenic variants and that the amino acid changes in these strains were similar to those seen in strains isolated in Victoria during 1995-1997. Therefore, IBDV strains in Australia show restricted geographic distribution, with classical strains prevalent in NSW, Qld and WA and variants prevalent in Victoria and SA. Given the highly infectious nature of IBDV strains, it is unclear why this restricted geographic distribution exists.

Phylogenetic analysis of 11 strains from this study, as well as 6 strains from the previous study, confirmed that Australian strains belong to two genetic groups, that of variant and classical strains. Both of these genetic groups are distinct from overseas strains. IBDV samples included in the study were from all major poultry producing areas in five states, thus providing a good representation of the type of IBDV strains occurring across Australia. Following the results of this study, it can be concluded with confidence that Australian strains can clearly be differentiated from all other overseas IBDV strains at the genetic level. This enables us to detect the incursion of any overseas strains into Australia, be it a vvIBDV, classical or variant IBDV strains.

The value of nucleotide sequencing for positive identification of local IBDV strains was confirmed in August 1999 during a suspected IBDV outbreak in NSW. The outbreak was initially diagnosed as being caused by vvIBDV strain based on histopathology. Samples from two farms involved were sent to AAHL for differential diagnosis which showed, by nucleotide sequencing and phylogenetic analysis, that IBDV strain involved was a classical like strain of Australian origin.

(4) Characterisation of antigenic and protective capacity of Australian IBDV variants

We previously showed during the 1995 - 1997 project on IBDV that commercial chicks with maternal antibody titres below 2,000 were not protected against challenge with variant strains. In this study we determined whether vaccination of dams with inactivated vaccines based on classical V877 and variant 02/95 strain influenced protection of progeny chicks.

Commercial layers were vaccinated in a laboratory environment with experimentally produced vaccines based on 02/95 variant and V877 classical strain. Antibody levels in progeny chicks were determined and chicks were challenged at 21 days of age with both classical V877, and variant 02/95 strain. The results showed that both vaccines produced similar antibody levels in progeny chicks. Chicks with maternal antibody titres above 3,200 were fully protected, regardless of the type of vaccine used. When antibody levels were in the range of 800 – 3,200 the type of vaccine used for vaccination of dams influenced the outcome of the challenge; chicks of dams vaccinated with variant strain were protected against both classical and variant challenge, whereas those from dams vaccinated with classical strain were only protected against challenge with classical strain. In the previous study we showed that commercial broilers with antibody titres above 2,000 (induced by vaccination of dams with classical vaccines) are protected against variant challenge. The requirement for a slightly higher ELISA titre of 3,200 (detected in this study) versus 2,000 (detected in the previous study) for full protection against all types of IBDV, might be due to differences in the vaccination regimes used in two studies. In this study commercial layers were not primed with live vaccines as is the common commercial practice. Further studies are needed to elucidate these differences.

Antigenic variants are also present in the USA. Although we previously showed that USA and Australian variants differ considerably at the antigenic and genetic level, it was of interest to assess protection afforded by USA vaccines against challenge with Australian variants. One USA vaccine containing a classical IBDV strain and one containing both classical and variant E strains, were used. Surprisingly, there was no difference in efficacy of protection afforded by these two vaccines against challenge with variant E. The USA bivalent vaccine also provided protection against challenge with the Australian variant 02/95, although the classical vaccine provided only partial protection against challenge with the variant 02/95. On the other hand, Australian vaccines based on classical or variant strains did not provide protection against challenge with variant E, indicating antigenic differences between the USA and Australian variants.

Results & Implications

Overall outcomes of this project are:

1. Generation of a reagent, Crab 88, specific for vvIBDV strains. Although we did not demonstrate that Crab88 could be used in a competitive ELISA, this antibody nevertheless is a valuable reagent. It can be used in an antigen ELISA significantly aiding in fast diagnosis of an exotic incursion of vvIBDV.

2. Confirmation that vvIBDV strains are prevalent in Indonesia and that they are almost identical to other vvIBDV strains. Thus all vvIBDV strains, regardless of the country of origin, are very similar and can be differentiated by nucleotide sequencing from all other IBDV strains.

3. Australian IBDV strains that are prevalent on commercial poultry sites are of two types, classical or an Australian variant.

4. Australia variant strains are prevalent in two states, Victoria and South Australia, whereas classical strains are prevalent in states of NSW, WA and Qld.

5. Australian IBDV strains are a unique group of strains that can be differentiated at the genetic level from all other overseas classical, variant and very virulent strains.

6. In progeny chicks, maternal antibody levels of 3200 are fully protective, regardless of whether dams were vaccinated with inactivated variant or classical type of IBDV.

7. In progeny chicks with maternal antibody levels of 800 – 3,200 full protection was dependent on the type of vaccine used; variant inactivated vaccine provided broader protection than classical inactivated vaccine. This finding suggests that commercial chicks with antibody levels in the range 800 – 3,200 might succumb to field challenge but only if variants are present.

8. Two USA inactivated vaccines, a monovalent containing a classical strain and a bivalent containing classical and variant E strains, provide protection against challenge with Australian variants.

9. However, Australian inactivated vaccines based on classical and variant strains did not provide protection against challenge with USA variant strain E, indicating antigenic differences between Australian and the USA variants. It is not clear from these results whether this one way cross- protection between US and Australian variants is due to antigenic differences between strains or due to other factors, such as for example vaccine formulation.

Publications

Ignjatovic J, Sapats S & Gould G. Characterisation of additional infectious bursal disease virus field isolates confirms existence of two distinct genetic groups in Australia. *Submitted for publication to Avian Pathology*

Ignjatovic J, Sapats S, Gould A, Boyle D, Gould G & Westbury H. Analysis of infectious bursal disease virus strains isolated from an outbreak suspected to be caused by exotic very virulent infectious bursal disease virus. *To be submitted to Australian Veterinary Journal*.

Ignjatovic J, Parede L H, Sapats S, Usman T, Soedijar & Gould G. Infectious bursal disease in Indonesia: characterisation of strains suggests high prevalence of very virulent strains. *To be submitted for publication to Avian Pathology*

Sapats SI, Heine HG, Trinidad L, Gould GJ, Foord AJ, Doolan SG, Prowse S. & Ignjatovic J. chicken recombinant antibodies against infectious bursal disease virus (IBDV) neutralises virus infectivity. *To be submitted for publication to Journal of Virology*.