

Development of infectious bursal disease vaccine and diagnostic kits for the control and prevention of the disease in poultry

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Introduction

Outbreaks of infectious bursal disease (IBD), an immunosuppressive disease of poultry with high mortality continue to occur despite of vaccination using commercially imported vaccines. Recently, fourteen local isolates of IBD virus (IBDV) were successfully isolated, adapted and propagated in SPF embryonated chicken eggs (CAM) and chicken embryo fibroblast (CEF) cell cultures. Some of the isolates had been characterized using both the conventional and molecular techniques, whilst the characterization of the others is still in progress. The pathogenicity of one of the selected isolate (UPM 93273) reduced after passages in CAM, however the immunogenicity of the isolate remained high and thus it is used for viral seed for the development of IBD vaccine. A sensitive and specific diagnostic kit is also vitally needs as a tool in the control and prevention of the disease. It was the objectives of the study to develop a safe and effective attenuated live IBD vaccine, and a sensitive and specific diagnostic kit using local isolates of IBDV for the control and prevention of the disease in poultry

Materials and Methods

The safety and efficacy of IBD viral seed (UPM 93273) which had been attenuated in CAM and CEF were determined. The viral seed was inoculated in various groups of commercial broiler chickens either at day 14 or days 14 and 28 of age. The chickens were monitored and any abnormal clinical signs were recorded. The chicks were sacrificed at various intervals, gross lesions were recorded and samples of serum were collected and analysed for IBD and Newcastle disease (ND) antibody titre using an ELISA techniques. The live weight and bursa of Fabricius weights of the chickens were recorded. The bursa of Fabricius was fixed in 10% formalin and process for histological examination and lesion scoring. On the development of the diagnostic kit reagents, selected IBDV isolates were purified, and inoculated into SPF chickens and rabbits at various intervals for hyperimmune serum production. The purified isolates were also cloned into bacterial plasmid vectors to be used for biotin-labelled probe and characterization of the IBDV. A serokinetic model of immune response was also determined using IBD antibody titre analysed from both the IBD vaccinated and non-vaccinated commercial layers and broiler chickens

Results and Discussion

A safe and effective attenuated IBD viral seed of local isolate was successfully developed following attenuation of the virus in embryonated chickens eggs (CAM). The seed virus showed highly immunogenic with low pathogenic when inoculated in commercial broiler chickens. The virus seed is ready for further test required for the production of IBD vaccine commercially. In contrast, the pathogenicity and immunogenicity of the IBDV local isolate losses following attenuation in chicken embryo fibroblast tissue culture (CEF). It is a need to developed IBD viral seed and vaccine based in tissue cultures as this will reduce the operational costs and shorter the production times. Various types of tissue cultures shall be tested to meet the above objective. It is important to note that the local isolates of IBDV were successfully characterised using both the conventional and molecular techniques as a very virulent (vv) IBDV of serotypes 1 which may have similar origin as those IBDV isolates in Europe and Japan. This further explains on the failure of IBD field vaccination, as all the imported IBD vaccines commercially available is an attenuated IBDV of the classical strains. It is an urgent need to developed IBD vaccine of vvIBDV to provide better protection against vvIBDV field challenged. The IBDV isolates were also successfully purified and IBD hyperimmune serum was successfully produced using both the SPF chickens and rabbits for the development of conventional and PCR based ELISA kits. The vvIBDV was found to be primarily replicate in the Harderian glands and gut associated lymphoid tissues following intraocular and oral inoculation, respectively leading to primary viraemia and replication of the virus in the bursa of Fabricius. Following the secondary viraemia, severe damages occurred in the bursa of Fabricius and other lymphoid tissues and vital organs leading to death or immunosuppression. The study also was successfully developed a serokinetic mathematical model of immune response to be used for determination of the right time and monitoring of IBD vaccination

Conclusions

The study was successfully develop a safe and effective IBD viral seed attenuated in chicken embryonated eggs (CAM) for commercial production of IBD vaccine. The local IBDV isolates was characterised as a very virulent (vv) IBDV of serotypes 1 which may have similar origin as those of IBDV isolates in Europe and Japan. IBDV reagent was successfully produced in rabbits and SPF chickens for the development of ELISA kits. The pathogenesis and serokinetic mathematical model of

immune response established in this study will definitely useful to be used in the control and prevention of the disease. Further study is required using various types of tissue cultures for the development of IBD vaccine based on tissue cultures

Benefits from the study

(1) Development of a safe and effective IBD viral seed for vaccine production commercially. (2) Development of IBD reagents to be used for ELISA kits. (3) Characterization of the virus as a very virulent (vv) IBDV of serotypes 1 using both the conventional and molecular techniques and thus provides vital knowledge on the possible origin of the virus and approach on the control and prevention of the disease. (4) Better understanding on the pathogenesis of vvIBDV. (5) Development of serokinetic mathematical model of immune response for IBD and thus will provide better approach in the control and prevention of the disease

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable

Up scaling of the IBD viral seed with local vaccine company for vaccine production commercially

Project Publications in Refereed Journals

- 1 Hair-Bejo M. 2002. Microscopy in R&D of infectious bursal disease in poultry in Malaysia. *Journal of Electron Microscopy Society of Thailand*, 16(1):39-41.
- 2 Phong SF, Hair-Bejo M, Omar AR and Aini I. 2002. Molecular characterization of Malaysian isolate of very virulent infectious bursal disease virus. *Asia-Pacific Journal of Molecular Biology and Biotechnology*, 10(2):89-96.
- 3 Hair-Bejo M, Salina S., Hafiza H, and Julaida S. 2000. *In ovo* vaccination against infectious bursal disease in broiler chickens. *Jurnal Veterinar Malaysia*, 12 (2): 63-69.

Project Publications in Conference Proceedings

- 1 Hair-Bejo M, Hafiza H, Phong SF, Omar AR, Aini I. 2000. Pathogenesis and pathogenicity of Malaysian isolates of infectious bursal disease virus. In: Proceedings of the 1st Joint Meeting of the Slovenian Society for Microbiology and the Hungarian Society for Microbiology, 2000 August 24-27, Keszthely, Hungary, p 2.
- 2 Hair-Bejo M, Chan KK, Wong CC, Hafiza H, Salina S. 2000. Safety and efficacy of feed based vaccine against infectious bursal disease in broiler chickens. In: Proceedings of the 12th Veterinary Association Malaysia Congress, 2000 September 1-4, Kuantan, Pahang, p 27-28.
- 3 Hair-Bejo M, Tee LW, Thu-Zar T, Salina S. (2000). Response of the bursa of Fabricius of chickens to an attenuated local isolate of infectious bursal disease virus. In: Proceedings of the 7th Asia-Pacific Electron Microscopy Conference - Life Sciences, 2000 June 26-30, Singapore, p 102-102.
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- 8 Thu-Zar T, Hair-Bejo M, Aini I. and Bahaman AR. 1999. Pathogenicity and immunogenicity of attenuated local isolate of infectious bursal disease virus seed for vaccine development in specific pathogen free chickens. In: Proc. National Congress on Animal Health and Production, September, 3-5, 1999, Alor Gajah, Malacca, Malaysia, p. 341-345.
- 9 Phong SF, Hair-Bejo M, Omar AR and Aini I. 1999. Outbreak of infectious bursal disease in vaccinated replacement layers. In: Proc. National Congress on Animal Health and Production, September, 3-5, 1999, Alor Gajah, Malacca, Malaysia, p.327-330.

Graduate Research

Name Graduate	of Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Phong Su Fun	Characterization of infectious bursal disease virus isolated in Malaysia for the development of diagnostic tools	Pathology and Immunology	PhD	2002

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