Review Article

Vaccine against Jembrana Disease Virus Infection: A Summary Findings

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Abstract: Jembrana disease constitutes the main concern in cattle industry especially in Indonesia and Australia as it has caused important economic losses due to mortality of cattle. The pathology of the disease is unusual for a lentivirus infection as it is associated with a severe, often lethal disease syndrome and a short incubation period in cattle. For lack of efficient medical treatment of JDV-infected cattle, vaccination may therefore constitute an effective measure for the prevention or eradication of Jembrana disease. Up to date, only one type of vaccine has been reported and tested. It is based on inactivated, tissue-derived virus antigens (Tabanan/87 isolate, $JDV_{TAB/87}$). This review summarize show current Jembrana disease vaccine was developed as well as evaluated and how these information might be useful in future vaccine design.

Keywords: Jembrana Disease, Vaccine, Prevention and Control, Bovine Disease, Immunization

Introduction

Jembrana disease was identified first in the Jembrana district of Bali island in Indonesia as an acute infectious disease (Kusumawati *et al*., 2014a). During the first observed outbreaks of Jembrana disease, the clinical disease was not reported in other types of cattle and this led to belief that the disease is unique to Bali cattle. Moreover, the mortality of more than 50000 cattles in a year was observed during the first outbreak (Budiarso and Hardjosworo, 1976). The disease is now endemic through-out parts of Indonesia (Hartaningsih *et al*., 1993) and also in Australia (Chadwick *et al*., 1998). Jembrana disease poses the major problem in Bali cattle industry especially in Indonesia and Australia due to the high mortality of the infected cattle, resulting in economic loss (Chadwick *et al*., 1998; Soesanto *et al*., 1990). The etiological agent, consequently named as

Jembrana Disease Virus (JDV). It belongs to the family of Retroviridae, of the subfamily of lentiviruses (Kusumawati *et al*., 2014a). The pathological process of JDV infection is quite different from other lentivirus infection as it is related with a short incubation period duration and a severe disease syndrome (Kusumawati *et al*., 2014b). Fatal issues, due to multiorgan involvement, occur only one to two weeks post-infection. These disease characteristics are in contrast to the chronic and progressive diseases over a long incubation periods typically associated with most lentiviral infections (Wilcox *et al*., 1995; Wilcox, 1997).

JDV-infected cattle exhibit a delayed and temporarily suppressed humoral response. This provides an explanation for the common occurrence of secondary infections which may lead to fatal issues in affected cattle (Dharma *et al*., 1991; Teuscher *et al*., 1982). The finding showed that no recurrence of disease is observed in

recovered animals and importantly they resist to further infections for at least 22 months (Dharma *et al*., 1991; Soeharsono *et al*., 1990). This suggests that JDV infections induce a protective immunity and probably vaccination using denatured or attenuated viral particles, can be performed to limit the disease spread. For lack of efficient medical treatment of JDV-infected cattle, vaccination may therefore constitute an effective measure for the prevention or eradication of Jembrana disease. Up to date, only one type of vaccine has been reported and tested (Ditcham *et al*., 2009; Hartaningsih *et al*., 2001). It is based on inactivated, tissue-derived virus antigens (Tabanan/87 isolate, JDV$_{TAB/87}$) (Ditcham *et al*., 2009; Hartaningsih *et al*., 2001). Presently, the vaccine is commercially available (JDVacc) and is routinely used for controlling the spread of Jembrana disease (Ditcham *et al*., 2009). This review deals with JDV vaccine which has been developed, summarizing its development, delivery, efficacy and what can be improved in future vaccine design.

Production of JDV Vaccine

Sources of Virus, Methods of Preparation and Adjuvants

During the acute phase, high titers of infectious virus are found in plasma and in certain organs such as spleen. They constitute therefore sources for virus production. Plasma-derived antigens were prepared from heparinized blood by low speed centrifugation then concentrated by high speed centrifugation. Spleen-derived antigens were prepared by homogenizing spleen of infected cattle, to provide a 10% spleen concentration and centrifuged at very low speed. Viruses were inactivated either by formaldehyde or Triton X-100. Two adjuvants were tested: Incomplete Freund's Adjuvant (IFA) or a Mineral Oil Adjuvant (MOA) and the vaccine preparation was emulsified to form a homogenous suspension (Hartaningsih *et al*., 2001).

Modes of Vaccine Delivery and Challenge

Vaccination was done by intramuscular inoculation (in neck region) with two or three doses at monthly intervals. The absence of febrile response is taken as an indicator of complete inactivation of virus. Challenge was carried out with virulent JDV$_{TAB/87}$ isolate (Chadwick *et al*., 1995; Soeharsono *et al*., 1990) one month following the last vaccine dose. Vaccination response was examined for clinical disease and rectal temperature. Normal criteria for the development of Jembrana disease were a concurrent febrile reaction and leucopenia of two or more days duration. Examination of gross and histopathological lesions of Jembrana disease was also performed at the conclusion of the observation period. Humoral

response was established by serological assays, using Western blotting (Hartaningsih *et al*., 2001).

Effects of Different Factors on Vaccine Efficiency

Their effects were examined and statistical results are considered as the overall response. Several factors may influence vaccine efficiency as described below (Hartaningsih *et al*., 2001).

Inactivating Agent

The effects of formaldehyde and Triton X-100 were examined on plasma-derived virus preparation. Viruses were completely inactivated by both inactivating agents as none of the vaccinated cattle developed a febrile response within 14 days after challenge. Animals, vaccinated with Triton-X100-inactivated viral particles, exhibited either partial or near complete immunity, with milder JDV-specific clinical symptoms following a challenge. Vaccination with formaldehyde-inactivated viruses also induced the production of JDV-specific antibodies though at markedly lower level but the vaccinated animals still developed clinical and haematological changes typical to Jembrana disease. It appeared so that Triton X-100-inactivated viral particles gave more satisfying result although the protective effect of the vaccine was not complete (Hartaningsih *et al*., 2001).

Adjuvant Efficacy

Comparison of the efficacy of IFA and a MOA was done using plasma-derived vaccine inactivated with Triton X-100. With both types of vaccine, three doses were required to reduce the duration of febrile state and the disease severity following a challenge. In necropsied cattle, the histological changes were considerably milder. The titers of antibody were similar in both cases and they progressively increased after each dose of vaccine. Antibodies to p26 (capsid protein) were the firstly detected whereas antibodies specific to other viral proteins were only detected after the third vaccine dose. Overall, similar effects were attained with either IFA or a MOA (Hartaningsih *et al*., 2001).

Influence of Tissue Origin

The developed JDV vaccines were either plasma- or spleen-derived. Comparison of their efficacy was done by performing vaccination with antigens inactivated with Triton X-100 and emulsified in MOA at monthly intervals. Statistically, examination of the typical clinical signs showed that the spleen-derived vaccines were more efficient than the plasma-derived ones as judged by a greater capacity in reducing the disease severity after challenge. Nevertheless, the level of JDV-specific antibodies was similar in cattle vaccinated with virus preparation from both origins (Hartaningsih *et al*., 2001).

Conclusion from Comparative Evaluation

From results obtained by comparing the effects of different parameters, it can be concluded that the optimal vaccination procedures against Jembrana disease are as follows: Inactivation of the virus in tissues with Triton X-100, use of a MOA and three times vaccination at monthly intervals.

Evaluation of JDV Vaccine

Pathology of Jembrana Disease

Understanding the pathological process in JDV infection is important as a basic knowledge for evaluating the effect of vaccine in the progression of the disease. Here we briefly describe the relevant pathological process in the progression of Jembrana disease. Clinically, the inoculated Bali cattle (with blood from an infected JDV cattle) exhibited several clinical signs after a short incubation period of 5 to 12 days, including lethargic state, anorexic state, hyperthermic state $\geq 39.3°C$ persisting for 7 days (rectal temperature), swollen of superficial lymph nodes, pallor in the mucus membrane, a mild ocular and nasal discharge and diarrhoea with fecal blood (Soesanto *et al.*, 1990). Laboratorically, the notified major changes in haematological condition included a normocytic normochronic anaemia, leucopenia (particularly lymphopenia, eosinopenia and a slight neutropenia), a mild thrombocytopenia, elevated blood urea concentrations and reduced total plasma protein (Soesanto *et al.*, 1990). Viruses are also detected in a high titer (up to 10^8 ID_{50}/mL, equaling to 10^{10} to 10^{11} viral genome copies/ml) in the plasma fraction of the blood during the febrile state (Soeharsono *et al.*, 1990; Soesanto *et al.*, 1990; Stewart *et al.*, 2005). The majority infected animals cultivate possible amount of antibodies to be detected only 6 weeks or more after recovery from the acute phase of the natural disease (Hartaningsih *et al.*, 1994). The temporary immunosuppression occurring during the acute phase was demonstrated by a decrease of IgG-containing cells in the lymphoid organs (Dharma *et al.*, 1994). However the ratio of IgG-containing cells raised during convalescence (Dharma *et al.*, 1994). Moreover, a study used Agar Gel Immunodiffusion (AGID) and Enzyme-Linked Immunosorbent Assay (ELISA) showed a slowed antibody response in JDV-infected animals (Desport *et al.*, 2009). Thus antibodies are not thought to be the major factor in the clearance of JDV in the acute phase as humoral immune response is produced several week after natural infection (delayed humoral response phenomenon). The comprehensive review of pathological process in Jembrana disease has been reported elsewhere by Kusumawati *et al* (2014b).

JDV Vaccine Efficacy

The spleen-derived JDV vaccine, JDVacc, is a detergent inactivated virus in homogenized spleen, inactivated by Triton X-100 and emulsified by a MOA. Two doses of JDVacc is the currently recommended vaccination regime. The protection provided is not complete, but the reduction of the duration and the disease severity and the infectious period may be sufficient to ameliorate the disease and hence to limit the transmission of virus to susceptible cattle (Ditcham *et al.*, 2009; Hartaningsih *et al.*, 2001). It has been shown that disease transmission possibly occurs when the virus titer is above a threshold below which the risk of disease transmission is highly reduced. In Jembrana disease, the threshold is thought to be 10^6 genome copies/mL in plasma, equivalent to 10^4 ID50/mL in plasma and a titer of $10^{10}-10^{11}$ genome copies/ml is currently observed during the febrile state (Soeharsono *et al.*, 1990; Soesanto *et al.*, 1990; Stewart *et al.*, 2005). Due to the high titers of infectious viral particles, virus transmission is most likely to occur during the acute phase. A vaccine that allows to reduce the duration and the magnitude of viraemia would also reduce the infectious period and the risk of disease transmission. It is therefore essential to evaluate the capability of JDVacc to efficiently reduce the virus titer and consequently the rate of the disease transmission (Ditcham *et al.*, 2009). For this purpose, Bali cattle were vaccinated with two doses of JDVacc, two and one month before challenge (Ditcham *et al.*, 2009). Analyses included determination of the genome copies by real-time RT-PCR as described earlier (Stewart *et al.*, 2005; Kusumawati *et al.*, 2015) and humoral response by Western immunoblot and ELISA using recombinant JDV proteins as previously described (Desport *et al.*, 2005). The vaccine efficacy was determined for different parameters as described below (Ditcham *et al.*, 2009):

Humoral Response

JDV-infected cattle frequently succumb to secondary infections (Dharma *et al.*, 1991), resulting from transient immunosuppression characteristic to Jembrana disease (Wareing *et al.*, 1999). In spite of this, vaccinated cattle still develop virus specific antibodies following infection. The examination of the humoral response showed that the IgG response varied following the antigen species. In vaccinated cattle, the seroconversion occurred at 10-25 days post challenge for p16 (matrix protein). Antibody specific to p26 (capside protein) was induced after the first dose of vaccine, increased after the second dose and the level is maintained after challenge. As for anti-TM (transmembrane protein) antibody, it was detected at

10-25 days post-infection. In non vaccinated animals, there was a delayed seroconversion to JDV p26 and TM at 56 days post infection instead of 10-25 days and none developed anti-p16 IgG (Ditcham *et al.*, 2009).

Clinical Response

Reduction of the duration and magnitude of febrile response and of the severity of leucopenia are correlates of protective effect of vaccine. This is actually attained with JDVacc. In vaccinated cattle, the duration of the febrile response upon challenge was reduced as compared to control animals and the period of moderate fever was also shorter (Ditcham *et al.*, 2009; Hartaningsih *et al.*, 2001). However the occurrence of febrile response induced at lower levels of virus in vaccinates highlights an opposite effect and shows a balance between effective immune activities and potentiation of viral replication (Ditcham *et al.*, 2009). These confounding effects of vaccination have also been observed in other lentiviral infections (Poli and Fauci, 1993; Richardson *et al.*, 2002).

Genome Copy Number

The threshold of 10^6 JDV genome copies/ml of plasma is indicative of a high level of viral replication hence of the probability of disease transmission to other animals. Therefore the period during which this threshold is attained, can be considered as the infectious period. All vaccinated animals had a viral load above the threshold level but its magnitude is much lower. Its duration was also reduced. It is thought that the reduction of the duration and of the severity of Jembrana disease and also of the infectious period may be sufficient to reduce the mortality rates and the transmission of virus to susceptible cattle (Ditcham *et al.*, 2009; Hartaningsih *et al.*, 2001).

Virus Clearance

The most significant effect of JDVacc is the faster virus clearance in vaccinates compared to control animals (Ditcham *et al.*, 2009) although the mechanism involved is not yet fully understood. The lower level of viral load may be due, at least partly, to the humoral response which enables virus neutralization. This will result in the reduction of early virus spreading. The combined elevated rectal temperature with lower virus titers and the effective virus clearance may suggest that the effective virus clearance results from a primed cell-mediated immunity response. This aspect has nevertheless been experimented.

Conclusion

Vaccine trials are not always easy to carry out correctly due to possible individual variations. Testing of potential JDV vaccines is facilitated by the acute nature of Jembrana disease, induced after a short incubation periods. Therefore long periods of monitoring are not required to assess the effects of immunization. The vaccine type which has been developed from spleen of JDV-infected cattle, JDVacc, is the sole that has been tested and presently commercially available and currently used to circumscribe the spread of Jembrana disease (Ditcham *et al.*, 2009; Hartaningsih *et al.*, 2001). Neutralizing antibody is likely not the essential mechanism in recovery from acute Jembrana disease as it is detected in animals only after a prolonged period following recovery from clinical disease (Hartaningsih *et al.*, 1994; Soeharsono *et al.*, 1990). Despite the lack of complete efficacy, the immune response attained in vaccinates appears to be sufficient to reduce the disease severity and the virus infectivity, all the more as naturally resistent Bali cattle will also help to limit disease outbreaks (Desport *et al.*, 2009). The JDVacc trials confirmed the fact that some degree of protection can be achieved against lentivirus diseases by whole virus vaccine, such as Feline Immunodeficiency Virus (FIV) (Bischop *et al.*, 1996; Diehl *et al.*, 1996), Equine Infectious Anaemia Virus (EIAV) (Wang *et al.*, 1994) and Simian Immunodeficiency Virus (SIV) (Vaslin *et al.*, 1994). The use of other types of vaccine, such as recombinant antigens and DNA vaccines, should be explored although some types of recombinant antigen cannot be correctly produced. Recently, a JDV-derived disabled, replicative-defective gene transfer vector has been developed (Metharom *et al.*, 2000; 2001; Olsen, 2001). It can express exogenous genes of interest but it can also be used as a delivery system for JDV gene products such as *gag* and *env* products which are difficult to produce in conventional expression plasmids. Such a delivery system can be used to produce important immunogenic antigens simultaneously with factors that enhance the induction of efficient immune response. Jembrana disease constitutes an important concern in cattle industry. As drugs specific to JDV are not available, in combination with suitable health measures and routine health controls, vaccination will constitute an efficient measure to prevent disease outbreaks and to reduce the consequent economic losses.

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Author's Contribution

Asmarani Kusumawati: Participated in all aquisition of literatures, reviewed the literatures, coordinated and contributed the writing of the manuscript.

Tenri Ashari Wanahari: Participated in all aquisition of literatures, reviewed the literatures and contributed the writing of the manuscript.

Pudji Astuti: Provided the technical guidance in literature review and contributed of the writing of the manuscript.

Kurniasih: Reviewed the literatures and provided the technical guidance in literature review.

Basofi Ashari Mappakaya: Contributed the writing of the manuscript.

Hastari Wuryastuty: Provided the technical guidance in literature review.

Ethics

This study was approved by Research Ethics Commitee, Faculty of Veterinary Medicine, Gadjah Mada University, Indonesia.

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