Recombinant Vaccine - A Vaccine Development and Antigen for Controlling Jembrana Disease in Bali Cattle

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Abstract. Most vaccines in Indonesia were initially made up from relying on attenuation or inactivation of pathogens. However, there have been developed several recombinant vaccines for poultry diseases. Advances in molecular biology, genomics and proteomics have facilitated in creating of vaccine development. Nowadays, vaccine can be developed by approach of recombinant protein technology. Jembrana disease is a viral and infectious disease and only attacks in Bali cattle. Up to present the vaccination of Jembrana disease has been conducted by using a crude vaccine which is made up from infected lymph origin and processed by ultra-centrifuge and emulsified with adjuvant. This crude vaccine has a short immunity for about 3 months. Breakthrough in molecular biology with approach of protein recombinant technology, Jembrana vaccine was developed in our laboratory of Animal Molecular Genetics of the Research Center for Biotechnology – LIPI. Jembrana disease virus (JDV) belongs to Lentivirus, it has three major genes termed as gag, pol and env. These genes encode proteins needed for replication of the virus. The env gene encodes TM and SU proteins located on surface of the virus and induce a protective immunity against lentivirus infections. Another protein is TAT which encoded by tat gene as one of small accessory genes located between pol and env genes. Two genes of tat and env su were originated from infected lymph by Jembrana virus and constructed into vector plasmid pET. Lately the two of tat and env su genes were synthetically made up and constructed into vector plasmid of pBT-7-His-C. Both nature and synthetic gene original constructions were transformed through Escherichia coli strain BL21. Both nature and synthetic tat gene original were already expressed in a small scale production of protein recombinant. The protein recombinant JTAT was first used in field trial for vaccine candidate and was examined by ELISA for jembrana disease detecting as diagnostic candidate. The result of both field trial and detecting Jembrana disease will be discussed for vaccine and antigen candidates, respectively.

1. Introduction
Availability of human or animal vaccines relies initially on mostly on attenuation or inactivation of pathogens. Advances in immunology, molecular biology, biochemistry, genomics, and proteomics could contribute in vaccine development and give new strategies in vaccination [1]. It has been reviewed that vaccination has proven to be the best strategy reducing costly budget for controlling a wide variety of infectious diseases in humans and animals [2]. It also happens in Australia that most expenses are budgeted for vaccination of internal parasite worm in sheep.

Up to present, most animal vaccines used in Indonesia also rely mostly on attenuation or inactivation of pathogens. However, there have been developed several recombinant vaccines for poultry diseases [3]. Those recombinant vaccines in poultry have been investigated for several diseases in poultry (Table 1). There are several disadvantages of such matter the conventional vaccine, such as low production, time consuming in production, shorter immunity and not targeting to the pathogens. The availability of
conventional vaccine could not fulfil the need of vaccine in the field when the animal grows quicker in population.

Table 1. Recombinant Vaccine for Poultry based on Vector Virus and Expressed Antigen [3]

| Vector Species | Vector Strain | Inserted Gene | Targeted Pathogen |
|----------------|---------------|---------------|-------------------|
| Pox virus      | Avipox virus  | H5, H7, +N1   | Avian Influenza Virus |
|                |               | HN, F,HN+F    | Newcastle Disease Virus |
|                |               | gM            | Marek’s Disease Virus |
|                |               | VP2           | Infectious Bursaid Disease Virus |
|                |               | env           | Spleen Necrosis Virus |
| Herpes virus   | ILT           | H5, N1        | Avian Influenza Virus |
|                | MDV           | F             | Newcastle Disease Virus |
|                |               | VP2           | Infectious Bursaid Disease Virus |
|                | HVT           | gM, RNAl      | Marek’s Disease Virus |
|                |               | HN, F, HN+F   | Newcastle Disease Virus |
|                |               | gA, gM        | Newcastle Disease Virus & |
|                |               | VP2           | Marek’s Disease Virus |
|                |               | H5            | Infectious Bursaid Disease Virus |
| Adeno virus    | Adeno virus   | H5, NP, M2    | Avian Influenza Virus |
|                |               | VP2, mRNA     | Infectious Bursaid Disease Virus |
|                |               | Fibre protein | Egg Drop Syndrome |
|                |               | S1            | Infectious Bronchitis Virus |
| Baculovirus    | Baculovirus   | H7, H5, N1    | Avian Influenza Virus |
|                |               | HN, F         | Newcastle Disease Virus |
|                |               | VP2           | Infectious Bursaid Disease Virus |
|                |               | VP1, VP2, VP3 | Chicken Anemia Virus |
|                |               | gI            | Gallid Herpesvirus3 |
|                |               | Antigen A (gp 57-65) | Marek’s Disease Virus |
|                |               | Capsin protein | RSS (Astroviridae) |
| Paramyxovirus  | NDV           | H5            | Newcastle Disease Virus |

A remarkable new generation of vaccines was developed recently this includes subunit, synthetic peptide and virus vector vaccines. Recombinant DNA technology has made it possible to insert and express heterologous genes in a variety of different viruses. Recombinant protein has been developed for recombinant vaccine for several diseases in ruminants. Single capripoxvirus recombinant vaccine has been long developed for cattle protection against rinderpest and lumpsy skin disease [4]. The rinderpest disease is no more counted in the world since 2011. A recombinant subunit vaccine was also developed for protection of *Histophilus somni* bacterial challenge in cattle [5]. So far, there is no recombinant protein vaccine developed for ruminant animals in Indonesia. This is a challenge for veterinary researchers to develop recombinant vaccines for several diseases against important economic pathogens either for dairy or beef cattle in Indonesia, such as Brucellosis, Mastitis and SE (*Septichaemia epizootica*).

2. Result and Discussion
2.1. Jembrana Disease

Jembrana Disease (JD) is a viral disease caused by Jembrana Disease Virus (JDV) and the most case in Bali cattle with mortality up to 20% [6]. The disease only attacks Bali cattle and initially occurred in Bali Island. There was outbreak in 1964 at Jembrana regency, Bali. Besides at Jembrana, the outbreak also occurred in 1964-1967 at Gianyar, Klungkung, Badung, Tabanan, dan Buleleng regencies of Bali [7]. However under experimental infection, mortality rate was reported about 17% based on field observations [8]. The JD was reported spreading up to Lampung, Bengkulu, South Sumatera, West Sumatera, Jambi, Riau, South, Central and East of Kalimantan [9]. The JD in West Sumatera was observed at Sijunjung regency [7]. The disease has short incubation period ranges of 5-12 days then following acute with clinical disease persists for up to 12 days [8]. The major clinical signs are not remarkable, i.e., fever, lethargy, anorexia and enlargement of the superficial lymph nodes [8]; (Margawati et al., 2017 unpublished technical report). Acute disease could sign of Hematohydrosis (blood sweating). It was reported that viraemic cattle could be for at least 2 years after infection and could be even for the life of animal this condition is therefore as a potential source of infection for other cattle [8].

Up to present, the availability of Jembrana vaccine is whole inactivated vaccine prepared from infected lymph and emulsified with adjuvant [10]. It more stated that the vaccine needs booster in its application to increase body resistant in the blood and vaccination should be conducted twice with interval a month. Availability of the inactive vaccine is also not consistent and not suitable to cover the need in the field as the Bali cattle increases in their population.

Breakthrough in advances molecular biology and this biotechnology tool the vaccine can be improved their quality with recombinant protein. Many genes of different original etiologic agents have been cloned, expressed and purified to be trialed as vaccines [1]. It was reviewed, it has been the last decade that veterinary vaccines have been substantially developed and demonstrated their effectiveness against many diseases [2].

Jembrana disease virus (JDV) belongs to Lentivirus, it has three major genes termed as gag, pol and env. These genes encode proteins needed for replication of the virus. The env gene encodes TM and SU proteins located on surface of the virus and induce a protective immunity against lentivirus infections. Another protein is TAT which encoded by tat gene as one of small accessory genes located between pol and env genes. Two genes of tat and env su were originated from infected lymph by Jembrana virus and constructed into vector plasmid pET [11]. Lately the two of tat and env su genes were synthetically made up and constructed into vector plasmid of pBT-7-His-C. Both nature and synthetic gene original constructions were transformed through Escherichia coli strain BL21. Both nature and synthetic tat gene original were already expressed in a small scale production of protein recombinant (Margawati et al, 2016, 2017, unpublished technical reports).

![Figure 1](image.png)

Figure 1. Characterization of JTAT protein in WB (Left) and SDS-PAGE (right) (E=Elusi; M= Protein Ladder, Precision Plus Protein Dual Xtra, BIORAD)
With regard of that condition, our laboratory of Animal Molecular Genetics of the Research Center for Biotechnology – LIPI has initiated to develop the Jembrana vaccine. Two recombinant proteins of JTAT and JSU are now available.

2.2. Protein Recombinant Production
A construct of Jembrana tat (then termed as JTAT) originally either from nature or synthetic gene was transformed into Escherichia coli strain BL21. The E. coli bearing JTAT clones were produced in 1,5L using a 2-L bioreactor. The JTAT protein was purified by using Ni-NTA Resin Agarose then characterized using SDS-PAGE and Western Blotting (WB), see Figure 1.

The JTAT protein recombinant was in the right sizes of 11.8kDa for JTAT protein ([11], [12], [13]. The protein was quantified by a Genequant (Amersham) and the yield average was 3.794 mg/ml per 1,5L production (Margawati et al, 2017; unpublished technical report).

2.3. First Field Trial for Jembrana Vaccine Development
A total of six female Bali cattle of approximately one and half year old from Nusa Penida of Bali were used. Three Bali cattle samples were vaccinated with JTAT recombinant in a dose of 500ug/ml/head (CB1, 2, 3), one Bali cattle as a safety vaccine was treated with double dose (2x500μg/ml) (CB4) and one Bali cattle as a control (unvaccinated) (CB5). Each vaccination, vaccinated group received a prime (V1) and four weeks later was repeated with the same dose JTAT recombinant vaccine (V2), see Table (2).

| Treatment                              | No of Cattle (head) | Code of Sample | Delivery |
|----------------------------------------|---------------------|----------------|----------|
| Vacc. 1+ Vacc. 2+ Challenge            | 3                   | CB1, CB2, CB3  | im       |
| Safety Test (double dose) +            | 1                   | CB4            | im       |
| Non-Vacc. + Challenge                  | 1                   | CB5            | im       |
| Jembrana Virus Donor                   | 1                   | CB6            |          |
| Termination of Cattle Trial            | 6                   |                | Incineration |

*im = Intra Muscular*

It resulted that body temperature at all vaccinated cattle samples (CB1, CB2, CB3 and CB4/double dose) showed at bellow 38°C (Figure 2). This figured that the vaccine candidate of JTAT could be categorized as a safe vaccine. Fever is categorized when the body temperature achieved 39.5°C or more [14].

Viral challenge was employed using infection dose of a 10³ID50 (Jembrana virus). Challenge was conducted to all cattle except donor cattle (Table 2). Body temperature of all challenged cattle showed fever at d-7 after challenge (Figure 3). This result described that the dose (500μg/ml/head) of vaccination (2x) did not give protection to the challenged cattle. It seemed that the dose could not trigger yet the antibody. This finding is similar to the field trial using crude vaccine in Bali cattle [15].
Viral challenge was employed using infection dose of a $10^3$ID$_{50}$ (Jembrana virus). Challenge was conducted to all cattle except donor cattle (Table 2). Body temperature of all challenged cattle showed fever at d-7 after challenge (Figure 3). This result described that the dose (500μg/ml/head) of vaccination (2x) did not give protection to the challenged cattle. It seemed that the dose could not trigger yet the antibody. This finding is similar to the field trial using crude vaccine in Bali cattle [15].

Based on observation during challenge, the given vaccine dose still created clinical symptoms of glandular enlargement (lymphodenopathy femoralis and brachialis). These clinical symptoms are agreed to another review [16]. While based on histopathology and leucopenia examination, all cattle samples were infected by Jembrana virus after challenge. It signed sub clinical symptoms (proliferation of lymphocyte cells outside of follicles and decreases leucocyte) that are specific for Jembrana disease. Those signs were agreed to another study in Bali cattle [17]. Even though, new (recombinant) vaccines are greatly demanded to effectively control newly- and re-emerging pathogens in livestock, however, development of veterinary vaccines is a challenging task [2]. It is due to a variety of pathogens, hosts, and the uniqueness of host-susceptibility individually.

2.4. Laboratory Trial for Recombinant Jembrana Antigen
Occurrence of the JDV cannot be detected clinically, therefore it needs to conduct early detection and monitor antibody titer to predict the precise time of vaccination. The disease detection based on immunoassay will help in identification of infected cattle either vaccinated or unvaccinated animals.
Antibody against JDV can be detected by using specific antigen. At the moment, recombinant antigen for jembrana disease virus (JDV) was developed by protein recombinant protein technology [11]. The tat gene of JDV genome (300bp) was constructed into vector plasmid, and expressed through E. coli. The JTAT protein recombinant was used as an antigen for detecting JDV by using immunoassay (ELISA). In this study, two types of micro plate ELISA (High Medium/HM and Medium Binding/MB) coated with JTAT recombinant antigen was designed to detect antibody response against JDV by applying serum samples of Bali cattle. Antibody Response against JDV by using JTAT recombinant was presented in Figure 4. The antibody titer of JDV from each serum samples in two types of Microplate ELISA were shown in Figure 2 5 (HB) and 6 (MB).

Based on ELISA assay (Figure 1), in the HB ELISA micro plate (Fig 1a) the green tosca color showed sharper than to the MB (Fig 1b). This finding indicates the diagnostics is better using MB plate. The microplate figures correlated to antibody titer measurement, the HB showed the same pattern to Fig 1., which showed 3 out (S8, S14 and S20) of 34 Bali cattle (8.8%) with s/p ratio of 0.56; 1.47 and 1.21, respectively. Those 3 cattle were suspected and infected by JDV. However, there were only two out of 34 samples (S14 and S20) in the MB ELISA plates with s/p ratio of 1.52 and 1.43, respectively. Antibody titer is quantitatively higher value that indicates more antibodies [18]. These findings suggest that JTAT antigen is potential be used to detect JD in Bali cattle population.
3. Conclusion
A small accessory \textit{tat} gene of Jembrana Disease Virus (JDV) were successfully constructed either into pET-6his tag naturally origin from infected lymph tissue by Jembrana virus and into pBT-7-HIS-C using synthetic \textit{tat} gene. Both constructs of using natural and synthetic \textit{tat} gene were successfully expressed through \textit{Escherichia coli} strain BL21 and produced in a 2-L bioreactor. First field trial of JTAT recombinant candidate vaccine using a single dose of 500μg/ml/head in Bali cattle showed a safe vaccine. However, the single dose could not trigger antibody against the Jembrana virus challenge of 10^3 ID50. This field trial study needs to employ with more doses of JTAT recombinant vaccine candidate in vaccination. The JTAT recombinant antigen proved sensitively to detect Jembrana disease in a lower serum dilution of 1:100 and using recombinant antigen JTAT in a lower dilution of 1:400. This recombinant Antigen is potential to be used for detecting Jembrana disease.

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