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# A Recombinant Subunit $HS_{ABA392}$ as a potential Vaccine for Haemorrhagic septicaemia disease in livestock

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**Abstract**. Haemorrhagic septicaemia (HS) is a major disease in cattle and buffaloes, caused by certain serotypes of *Pasteurella multocida*, mainly B and E serotypes. Frequent HS outbreak has a major impact in many Asian countries, including Malaysia, where farmers encounter economic loss due to low milk production as well as death of their livestock. There are four types of vaccines available; broth bacterins, alum precipitated vaccine, aluminium hydroxide gel vaccine and oil adjuvant vaccine (OAV), but these vaccines can only provide short term immunity and therefore need to be administered annually. Hence, the development of a protein vaccine using recombinant antigen can be a potential candidate for the production of HS vaccine that would give longer immunity. We have successfully cloned the ABA392 gene fragment into a protein expression vector, pET-30a. The protein was expressed from our ABA392/pET30a clone and the immunogenicity of the protein has been tested on rats. This vaccine was able to trigger an immune response and therefore has the potential to be tested as suitable vaccine candidate in future studies. It is envisaged that this subunit vaccine will make a significant contribution in the management of HS among livestock in future.

## INTRODUCTION

Haemorrhagic septicaemia (HS) is an acute and fatal in cattle and buffaloes as these animals are more prone and vulnerable to this disease (Elshemey & Abd- Elrahman, 2013). There are large cattle and buffalo farms in Asia and HS is endemic, therefore it is important to prevent and control this infection in the livestock (Ammarah & Aamira, 2016 and Benkirane & De Alwis 2002). In Malaysia an average of 360 heads and buffaloes die due to the infection (Saad, 2013)). HS outbreak was confirmed through post mortem among buffaloes and cattle in West Bengal. A total of 154 animals were affected by HS whereby 52 of it died before the treatments were begin (Mitra *et al*., 2013). Meanwhile in Egypt, a total of 88 *P. multocida* were isolated from 256 nasopharyngeal and lung tissue of dead calves from January 2013 to March 2014.

*P. multocida* is a gram negative bacterium that causes HS and classified into five different serotypes which are known in the live population of different geographical distribution. Each serotypes causes different infection (Boyce *et al*., 2000). Serogroup A and D causes various infection in domestic animals and can be found worldwide (Jaglic *et al*., 2005) while serotype B and E are related with HS in Asia and Central Africa respectively (Qureshi and Saxena, 2014). In mean time, serogroup F is known for causing fowl cholera and commonly found in North America (Jaglic *et al*., 2005).

HS infection leads to severe depression, pyrexia, submandibular oedema, dyspnea and death (Horadagoda *et al*., 2001). Host is

infected through the inhalation and ingestion of the bacterial agent resulting in septicaemia and multiple-organ haemorrhages. Transmission of *P. multocida* is through gastrointestinal tract. However, urinary tracts can also be the possible route for the HS transmission (Annas *et al.*, 2014).

HS can be treated using antibiotics during the early stage of the infection but recent transformation of *P. multocida* against antibiotic sensitivity leads to a higher rate of morbidity and mortality (Shivachandra *et al.*, 2011). In order to prevent HS, vaccination of the vulnerable populations have been carried out. There are few types of vaccines such as killed vaccine, live vaccine, and combined vaccines, experimental anti-idiotype vaccine and autogenous vaccine.

The development of good vaccines against HS is highly desirable. Potential virulence genes in *P. multocida* can be targeted for protein subunit vaccine production with the expectation to provide long immunity. Recombinant clone ABA392 was derived from *P. multocida* serotype B202 through shotgun cloning method and possessed a sequence that encoded for a virulence factor in *P. multocida* (Salmah, 2000 & 2004). Since recombinant clone ABA392 might harbour a gene that has the capability of triggering the immune system and protects it from HS, the aim of this study is to evaluate the immunogenic potential of recombinant protein from ABA392-pET30a using different expression vectors in animal.

## MATERIAL AND METHODS

### Transformation of ABA392 Gene

The recombinant clone ABA392 (Salmah, 1997) was provided by Professor Dr Salmah Ismail, Molecular Bacteriology and Toxicology laboratory, Faculty of Science, University of Malaya. The bacterial strain *Escherichia coli* BL21 (DE3) pLysS was obtained from Invitrogen, USA. To clone the targeted gene, both ABA392 gene and pET-30a vector was double digested with restriction enzyme *Hind*III-HF and *Bam*HI-HF (New England Biolabs, U.S.A). Digested ABA392 and linearized pET-30a vector were purified following the protocol of Nucleospin Gel and PCR Clean Up (Macherey-Nagel, Germany) and ligated using T4 DNA ligase (New England Biolabs, U.S.A) thus recombinant clone ABA392-pET30a was constructed (Sambrook & Rusell, 2001). The ligated plasmid was then transformed into *E. coli* BL21 (DE3) pLysS competent cells. Resulting clone were grown in Luria Bertani (LB) media (Liofilchem Diagnostic, Italy) which supplemented with kanamycin (30µg/ml) and chloramphenicol (34µg/ml) (Sigma-Aldrich, U.S.A).

### Colony Library and Colony Polymerase Chain Reaction Chain (PCR)

The positive colony recombinant clone ABA392-pET30a was patch plated and grown overnight at 37ºC. The ABA392 gene from positive recombinant clone was further confirmed via using colony PCR by amplifying the gene by using designed primer specifically. The amplification mixture (25µl) of PCR contains each primer at a concentration of 10p/mol, 2X Econotaq plus green master mix (Lucigen, U.S.A), 4µl of extracted DNA sample and distilled water to make it as final mixture. The thermal cycling parameters included initial denaturation at 95°C for 2 minutes, 30 cycles of 95°C for 30 seconds, annealing at 64°C for 1 minutes, extension at 72°C for 30 seconds and final extension at 72°C for 10 minutes using thermal cycler machine (BIO-RAD, U.S.A). The amplified ABA392 gene sequence was confirmed by 0.7% agarose gel in Tris-Boric-EDTA (TBE) (Thermo Scientific, U.S.A) buffer run at a constant current of 70V. The gel was stained with gel red and viewed under UV trans-illumination.

### Isolation of ABA392-pET30a and DNA Sequencing

A positive colony was subjected to DNA isolation using the prescribed protocol of the Nucleospin Plasmid (Macherey-Nagel, Germany). The purified ABA392-pET30a was subjected restriction enzyme analysis using *Hind*III-HF and *Bam*HI-HF to verify

the inserted gene in the clone. Followed by DNA sequencing to re-confirm the presence of the desired gene.

**Expression and Purification of Recombinant ABA392-pET30a**

*E. coli* BL21 (DE3) pLysS cells that carrying ABA392-pET30a clone were incubated overnight on shaking incubator at 37ºC in 10ml LB medium (Liofilchem Diagnostic, Italy) containing kanamycin (30µg/ml) and chloramphenicol (34µg/ml). An amount of 10ml overnight culture was inoculated into pre-warmed 50ml LB medium and grown with vigorous shaking at 37ºC. Expression was induced at $OD_{600}$ of 0.6 by adding Isopropyl-β-D-Thiogalactopyranoside (IPTG) (Promega, U.S.A) to final concentration of 1mM at 37ºC for 4 hours in shaking incubator at 200rpm. Cells were than harvested (centrifugation) at 2500 x g at 4ºC by and pelleted cells were stored at -20ºC overnight prior to purification. The expressed cells were lysed using 4ml/gram of B-PER bacterial extraction reagent (Thermo Scientific, U.S.A) and the suspension was pipetted to obtain a homogenous solution. The solution was incubated for 15 minute at room temperature. The cell lysate was centrifuged for 5 min at 15000 x g to remove all the cellular debris (Noor Masyitah Jumahat *et al*., 2015). The supernatant containing expressed His-Tag recombinant protein was purified using Dynabeads His-Tag Isolation and Pulldown) (Thermo Scientific, U.S.A) according to the manufacturer's instructions. The purification technique is based on cobalt-based Immobilized Metal Affinity Chromatography (IMAC) which specifically used to purify histidine-tagged using magnetic separation. The purified proteins were separated via 12% Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique (Laemmli, 1970) using Mini Protean Tetra System (Bio-Rad, U.S.A) to analyse the protein. Protein samples were heated at 95ºC for 5 minute and electrophoresis was conducted at constant voltage 120V for 75 to 85 minute. Finally the 12% resolving gel was stained with Colloidal Coomasie Staining which composed of 0.08% Coomasie Brilliant Blue (CBB) G250 ( Bio-Rad, U.S.A), 1.6% ortho-phosporic acid, 8% ammonium sulphate and 20% methanol to show the separated proteins.

**Western Blot Analysis**

Eluted His-Tag proteins were run on 12% resolving SDS-PAGE and transferred to nitrocellulose membrane (0.45µm) using transfer buffer. Following this, the membrane was blocked. Western blot analysis was carried out by using Penta Anti-His Antibody, BSA free (Qiagen, Germany) as primary antibody at a dilution of 1:5000 (diluted in 2.5% skim milk in Tris-Borate Saline, pH 7.5) meanwhile, anti-mouse IgG, AP-linked antibody (Cell-Signaling, U.S.A) served as secondary antibody at a dilution of 1:5000 respectively. Washing steps with TBS-Tween 20 (TBST) were carried out and the targeted band was detected by 5-Bromo-4-Chloro-3-indolyl phosphate (BCIP) AP substrate (Merck U.S.A).

**Animal Experiment**

Female Sprague Dawley rats weighing approximately 150g were used in animal experiment. The preparation of bacterial suspension for vaccine was adapted from (Jamal *et al*., 2012) with slight modification. This bacterial suspension serve as positive control. The rats were housed casually in an individual cage at the experimental room, animal house, University Malaya. All the rats were immunized subcutaneously with the proper amount according body weight (Diehl, 2001). To determine the immunogenicity of the expressed recombinant protein from ABA392-pET30a, rats were immunized with two different concentration of the protein. Concentration of the protein was determined by using DC Protein Assay (Bio-Rad, U.S.A). For the positive and negative readings, rats were immunized with killed bacterin concentration of $10^7$ as mentioned above and 1X Phosphate Buffer Saline (PBS), pH7.4. The vaccination was carried out with double dose where 1$^{st}$ immunization was scheduled on day zero (week 1) followed by a booster dose 14 days post immunization which falls on week 3. All the immunized rats including

positive, negative and treatment groups were bled on day 0, 7th, 14th, 21st, 28th and 28th. The blood was collected: serum separated, pooled and stores at -20°C until it was used for ELISA. At the end of the vaccination, rats were sacrificed by overdose anaesthesia based on the ethic requirement. Animal experiment was performed under the approval of the Institutional animal care and use committee (IACUC), University of Malaya (approval ref no. ISB/18/11/2015/RDVM (R). Organs from the sacrificed animals were collected at end of the experiment for gross and histopathological analysis. Tissue processing was done manually, started with dehydration, tissue infiltration, embedding and sectioning. As the final steps, the tissue sections were stained with Harris hematoxylin and eosin Y (H&E) (Sigma-Aldrich, U.S.A) for further analysis.

**ELISA**

ELISA was developed based on the procedure by Okay *et al.* (2012) with slight modification. ELISA was performed on the hyper immune sera which obtained from immunized rats to detect the presence of antibody. Summarily, 96 well microtiter plate was coated with coating antigen (*P. multocida* antigen diluted in 0.05M carbonate-bicarbonate buffer, pH 9.6) and blocked with blocking buffer (PBS-Tween 20-BSA, pH 7.4). Serum sample was added at 1:50 dilution and incubated at 37°C for 1 hour. After completion the washing steps, IgG antibody (HRP) conjugate (Gene Tex, U.S.A) was subsequently added to the well with optimized ratio 1:2500 and incubated at 37°C for 1 hour. The plate was washed and tetramethylbenzidine (TMB) substrate (Nacalai Tesque, Japan) was added and incubated at RT for 30 minute. Finally, the reaction was stopped with 2M sulphuric acid (Fisher Scientific, U.S.A) and absorbance was read at 450nm on a microtiter plate reader.

**Statistical Analysis**

Independent T-test was used for mean comparison of antibody between group by using SPSS version 20 (Salmah Ismail *et al.*, 2012 and Singh *et al.*, 2011). The significance level (p value) was set at 0.001, SD calculated using SPSS version 20.

## RESULT

**Cloning ABA392 Gene into Expression Vector and Purification of Recombinant Protein from ABA392-pET30a**

The ABA392 gene was successfully cloned into an expression vector pET-30a and known as ABA392-pET30a. The clone size was 6.2 kb (Figure 1(a)) on the agarose gel electrophoresis analysis using supercoil ladder. Double digestion of the recombinant clone was carried out using the restriction enzyme H*ind*III-HF and B*am*HI-HF. This RE analysis will generate the clone into two fragment, an insert at the size of 804 bp and the vector size approximately 5422 bp (Figure 1 (b)). The polyhistidine-ABA392-pET30a was expressed and have an approximated size of about 32 kDa on 12% SDS-PAGE (Figure 2 (a)). Meanwhile, the size of recombinant protein from ABA392-pET30a was confirmed via immunoblotting by using Penta Anti-His Antibody (Figure 2 (a)).

**Vaccinated Rats**

Sprague Dawley rats were inoculated SC with 50µg and 100µg his-tag expressed protein from ABA392/pET30a. The sera were collected from the rats on day 0, 7, 14, 21, 28 and 35 and used to measure of IgG titer. As shown in Figure 3, once the primary immunization was given on 1st week, no development of IgG was observed until the second booster was given on 3rd week and the graph shows an increase in the IgG level of all groups expect for the negative control group. Serum level of the rats which treated with killed bacterin thus serve as positive control have also develop IgG from week 3 towards week 6. Serum level in rats vaccinated with expressed protein from ABA392-pET30a (50µg and 100µg) shows significantly induced higher antibody titers compared to negative group, 0.099, 0.0081 and 0.045 respectively with $p$ 0.001. Immune response of the expressed recombinant protein from ABA392-pET30a was achieved

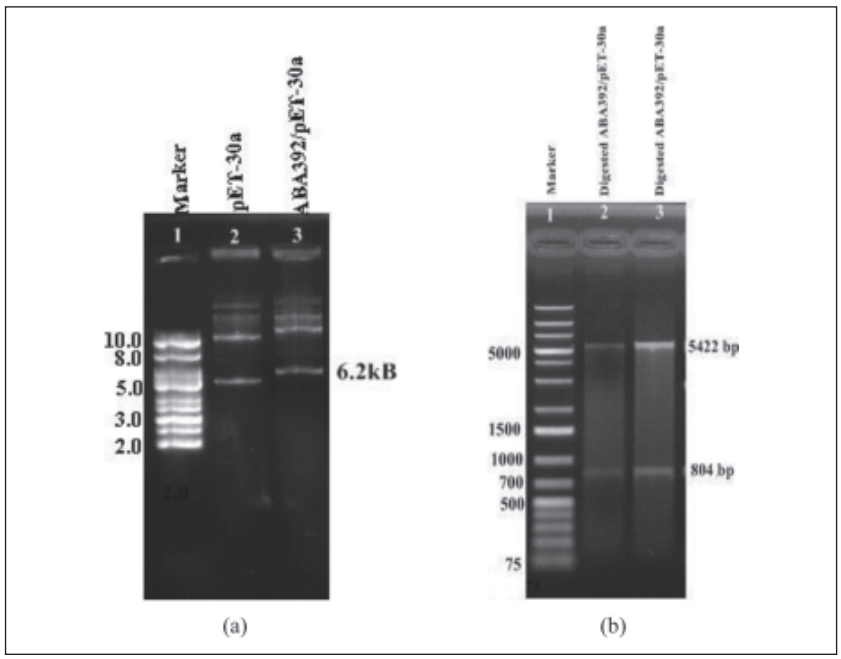


Figure 1. (a) DNA isolation of recombinant plasmid ABA392-pET30a. Lane 1, Supercoiled DNA ladder; lane 2, purified pET-30a vector (control) and lane 3, isolated ABA392-pET-30a recombinant plasmid. (b) Digested of ABA392/pET30 through restriction enzyme analysis using H*in*dIII-HF and B*am*HI-HF. Lane 1, Gene Ruler 1 kb plus ladder and lane 2 to 3, digested ABA392/pET-30a. The digested ABA392 shows size at 804 bp and pET-30a vector show size at 5422 bp.

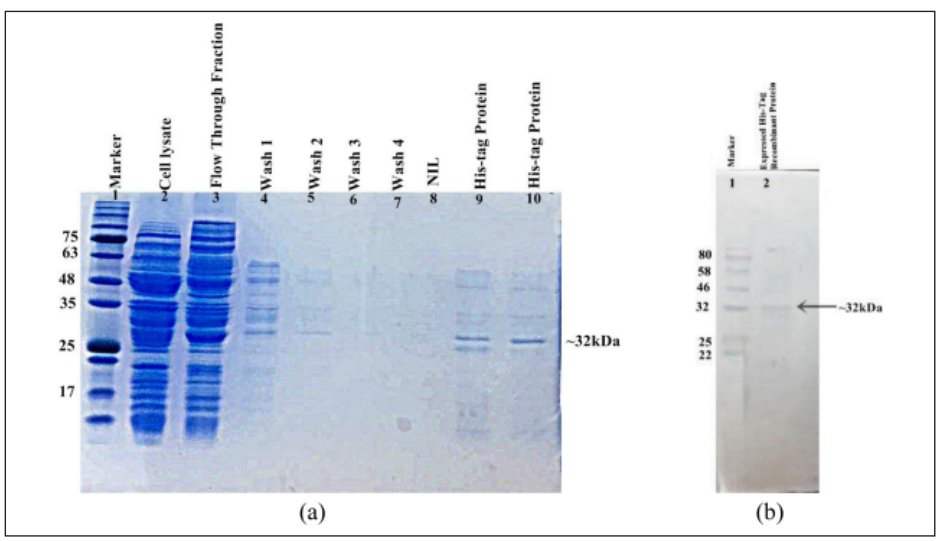


Figure 2. (a). Analysis of ABA392-Pet30a 6xHis-tagged protein expression in *E. coli* BL21 (DE3) pLysS using Dynabeads His-Tag Isolation and Pulldown. Lane 1, BLUeye prestained protein ladder; lane 2, cell lysate; lane 3, binding protein, lane 4 to 7, wash fraction 1-4 and lane 9-10, extracted his-tag recombinant protein approximately ~32kDa. The transformed ABA392-pET30a plasmid was induce with IPTG thus protein were expressed. The expressed was purified through affinity chromatography by using Dynabeads His-Tag Isolation and Pulldown and size were determined from SDS-PAGE, approximately ~32kDa. (b) Western blot analysis with Penta Anti-His Antibody. Lane 1, Protein prestained ladder and lane 2, expressed His-Tag recombinant protein.

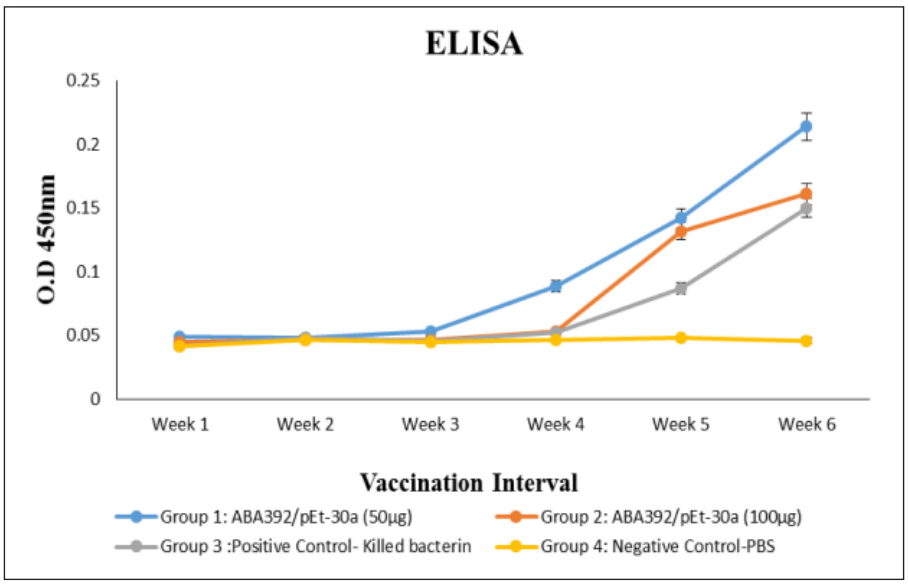


Figure 3. ELISA O.D analysis using 450nm. Comparison on serum IgG from rat vaccinated with purified protein from ABA392/pET-30a (50µg and 100µg) compared with rat vaccinated with normal saline and killed bacterin *P. multocida* serotype B. Each points shows mean of serum activities of three pooled serum from three individual rats (n=3) and bar represents SE value ($p<0.001$).

Table 1. No. of vaccinated rats with killed bacterin of *P. multocida* Serotype B as positive control, purified protein from ABA392-pET30a and normal saline as negative control vaccination and survival rate after the second booster

| Type of Immunization | No. of Dosage | No. of rats survived | Survived (%) |
|---|---|---|---|
| Purified protein from ABA392/Pet-30a (50µg) | 2 | 3/3 | 100 |
| Purified protein from ABA392/Pet-30a (100µg) | 2 | 3/3 | 100 |
| Killed bacterin of P.multocida Serotype B | 2 | 3/3 | 100 |
| Normal saline | 2 | 3/3 | 100 |

with two dose vaccination (Table 1) and showed to be effective in developing antibodies.

**Histopathology Analysis**

Histopathological and gross pathological studies were carried out on the organs of both groups, ABA392-pET30a and negative control groups. Upon the gross pathological examination, there is no significant changes were noticed on all the organs. Lungs, heart, liver and heart were examined and no lesion were found. Nucleus appear to be normal and slight thickening wall of alveoli with presence of red blood cell (RBC) can be observed in lung samples (Figure 4 (a) and (b)). Cardiac muscle cell of heart samples shows branches with normal looking nucleus (Figure 4 (c) and (d)). Liver sample from treatment and negative groups have a normal central vein and nucleus cells (Figure 4 (e) and (f)). Kidney sample of vaccinated with purify protein from ABA392-pET30a shows distal convoluted tubule with normal appearance (Figure 4 (g)) meanwhile glomerulus and proximal convoluted tubules appear to be normal in kidney sample of negative control group (Figure 4 (h)).

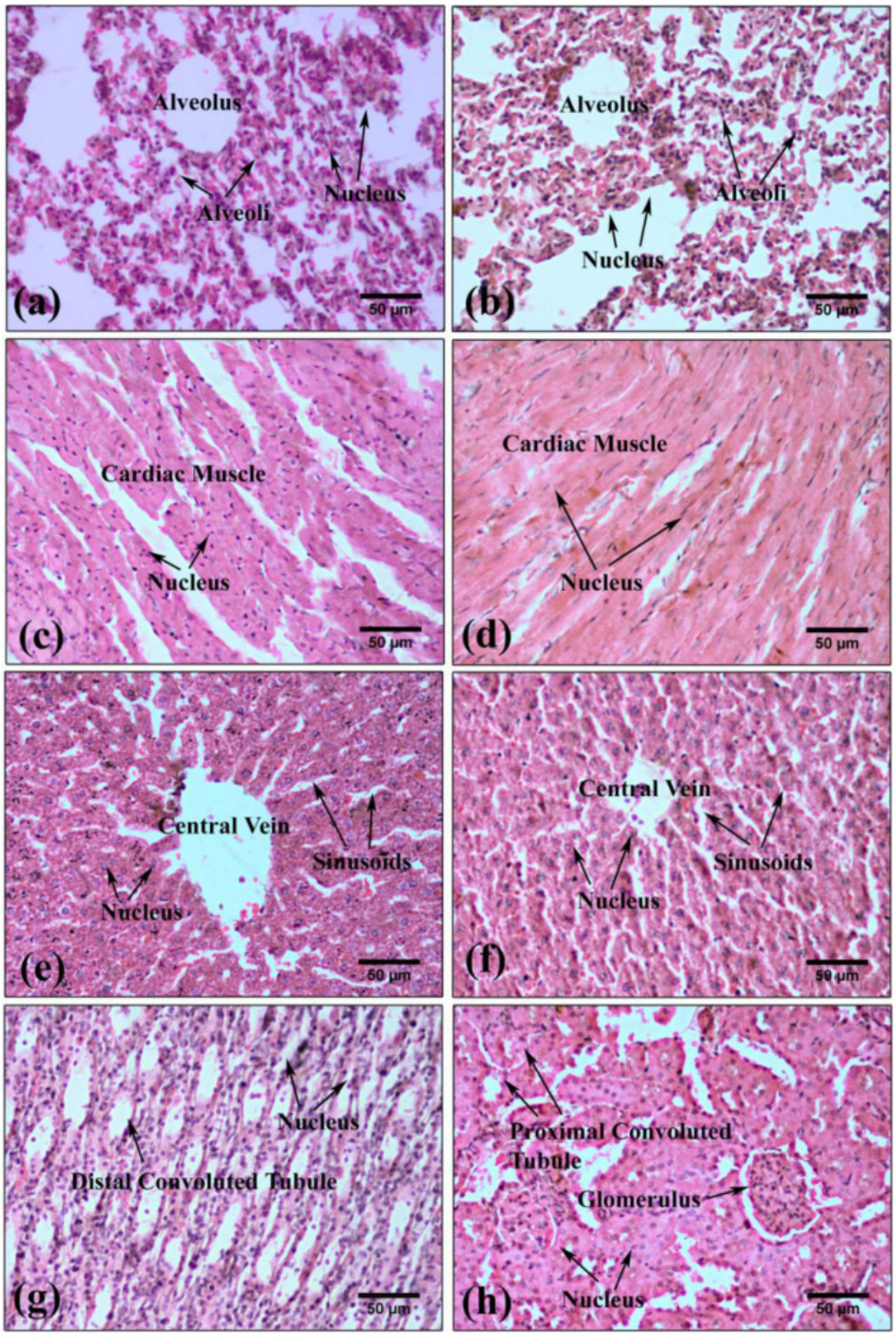


Figure 4. Photomicrograph of lungs, heart, liver, and kidney samples vaccinated with ABA392-pET30a and PBS (negative control) stained with Haematoxylin and Eosin (H&E). Histopathological section of the lungs (a), heart (c), liver (e) and (g) from rats vaccinated with purify protein from ABA392-pET30a with 20x magnifications, 50µm. Histopathological section of the lungs (b), heart (d), liver (f) and (h) rats vaccinated with PBS (negative control) with 20x magnifications, 50µm. No sign of inflammation or infiltration with inflammation cells or sign hemorrhagic were observed in all the sample.

## DISCUSSION

The first HS outbreak reported in Malaysia was in 1900 which caused huge lost to the meat and dairy industry (Corrongean, 1902). HS outbreak causes tremendous economic impact to the industry involving cattle and buffaloes, with an estimated RM2.4 million loss annually (Benkirane & De Alwis, 2002). The outbreak of HS in the peninsular of Malaysia over the years causing high mortality due to the lack of vaccination and inefficient veterinary services (Bisht *et al.*, 2006). Recombinant protein produced by *P. multocida* triggered strong immunity (Hatfaludi *et al*., 2010). Recombinant technology is reforming the shape of current vaccine development against *P. multocida* infection (Ahmad, 2014). From the previous results, recombinant clone ABA392 might harbor a gene that has the capability for triggering the immune system of the administrated host and protects it from HS disease (Jamal *et al.*, 2012). Hence, it was found that the recombinant clone ABA392 has an immunogenic factor and could be used as vaccine in future. Since the HS determinant is yet to be reported or discovered elsewhere, it is of great interest to conduct this study with the aim to evaluate the immunogenic potential of recombinant protein ABA392 using different expression vector.

In this study, ABA392 gene was successfully ligated with pET-30a vector and transformed into *E. coli* BL21 (DE3) pLysS system. The successfully recombinant clone known as ABA392/pET-30a and it was analysed in 1% agarose gel and the result showed the recombinant clone ABA392/pET30a at the size of 6.2 kB (Figure 1 (a) ). The RE analysis reconfirmed the insert and vector size of the recombinant clone ABA392/pET30a at 802 bp and 5422 bp respectively (Figure 1 (b)). The pET-30a expression vector was chosen due to the T7 promoter, kanamycin resistant, and multiple cloning sites and this vector is more applicable for the expression of recombinant protein in *E. coli* purpose. When the interested gene is cloned behind T7 promoter it will recognized by T7RNA polymerase (T7RNAP) (Rosana & Ceccarelli, 2014). Subsequently, presence of IPTG will induce and produce T7 RNA polymerase in *E. coli* thus prompt protein expression. T7 RNA polymerase will transcribe the particular coding sequence for the interested protein under the control of T7 promoter (Briand *et al.*, 2016). Beside this, sequences that encoding six histidine presence at 5' of the multiple cloning sites (MCS) is one of the significant reason on choosing pET expression vector in this study (Fang, 2006). *E. coli* BL21 (DE3) pLysS known as lysogenic bacterium that has λ-DE3 which responsible for encoding T7RNA polymerase under the control of the lacUV5 promoter. Meanwhile, the compatible plasmid pLysS carries the gene encoding T7 lysozyme which reduces the background expression level of the targeted gene under the control of T7 promoter without interfering with the level of expression achieved through IPTG induction (Rosano & Ceccarelli, 2014).

6x His-tag has been used extensively to purify recombinant protein due to applied a low metabolic burden on expression host and provide flexible condition for IMAC (Dan *et al.*, 2009). Cobalt-based Immobilized Metal Affinity Chromatography (IMAC) has the ability to display less non-specific protein binding compare to nickel and due to this it has increased the purity of eluted protein (Bornhorst & Falke, 2000). The expression of recombinant protein from ABA392/pET30a was successfully extracted, purified and confirmed via immunblotting and from the SDS-PAGE analysis, a 32 kDa protein band was determined (Figure 2 (a)). Immunoblotting was carried out in order to confirm the expression of 6xHis-tagged of ABA392/pET-30a. Transferred eluted his-tagged protein of ABA392/pET-30a reacted positively with Penta Anti-His antibody and anti-mouse IgG, AP-linked antibody. A band was visible at ~32 kDa size on the film was observed (Figure 2 (b)).

Immunogenicity of expressed protein of ABA392/pET-30a (50µg and 100µg) was distinguished. The vaccinated rats with

expressed protein from ABA392/pET-30a (50µg and 100µg) shows significantly induced higher antibody titers compared to PBS (negative control) and formalin killed bacterin (positive control) (Figure 3). Further, it was observed from primary vaccination with ABA392/pET-30a, a significant increase can be seen only after week 3 when second booster was given. It shows that IgG response is more rapid and significant by 14 day after the second booster is given on week 3 (Hodgson *et al.*, 2005). The increased of antibody can only be seen after the second booster were given which begin after week 3 and started to increase for the next two weeks Even though administration of recombinant protein vaccine has the capability to prompt immune response against pathogen after being expressed by plasmid or harmless bacteria but it sometimes shows a weak or low immune response. Thus, it sometimes requires the usage of an adjuvant in order to provoke protective and last long term immunity. The main problem encountered during development of new adjuvant is not only involving in understanding their molecule complexity yet also the mechanism of the immune response activation (Nascimento & Leite, 2012). The same observation was made by Yasin *et al*. (2011), when using the expressed recombinant protein vaccine from fimbrial protein of *P. multocida* B: 2 whereby, the antibody level gradually rise and increased significantly for the next 14 days after the booster dose was given. This might be due the primary vaccination was unable to boost the memory cells to produce a significant level of secondary antibody thus a booster dose was required (Yasin *et al*., 2011). Another possibilities why a booster dose is required, because the vaccine might be degraded rapidly by the host defence mechanism without giving the memory cells to copy the vaccine material (Isaguliants *et al*., 2004). Furthermore, significant difference was observed among sera from rat vaccinated ABA392/pET-30a (50µg and 100µg) compared to negative group ($p<0.001$) which able to provoke humoral immune response against recombinant plasmid (Singh *et al.*, 2011; Salmah Ismail *et al*., 2012). Sera from formalin killed vaccine have been observed and show the significance difference when compared to the negative control. This is due to the formalin killed vaccine have the same homologus potency as inactivated vaccine. The immunity may have obtained half from host specific and bacterium itself which cause ineffective and short immunity thus became the disadvantage of this killed vaccine. Recombinant vaccine which known as third generation vaccines and has specific aspect in producing vaccine against *P. multocida*. Identified virulence and immunity gene from *P. multocida* has a promising in the recombinant protein vaccine production (Ahmad *et al*., 2014).

Histopathological studies were performed on both groups of ABA392/pET-30a and negative control groups. Upon gross pathological examination, there were no significant changes were noticed on the site of injection of ABA392/pET-30a on the vaccinated rats. Based on the histopathology analysis, there were no sign of HS like haemorrhage, inflammation cells, oedema or hyperaemia were observed in the lung, heart, liver or kidney tissue (Figure 4 (a), (c) (e) and (g)) (Chung *et al*., 2015). This finding signifies that ABA392/pET-30a protein vaccine does not cause any visible changes to the host and it can be suggested safe to use as a vaccine since it has not shown any toxicity effect on the tissue or the site of administration.

The use of DNA recombinant technology such as gene cloning and recombinant protein production from *P. multocida*, could trigger immune responses and provide protective immunity to the administered animal. Potential virulence genes in *P. multocida* can be targeted for protein subunit vaccine production. The first successful attempt was against rhinitis in pigs which was derived from *P. multocida* toxin (PMT) (Bording *et al*., 1994). *TbpA* a gene that encode transferrin binding protein from *P. multocida* serogroup B: 2 have expressed high antigenic recombinant protein which has the potential in developing vaccine against HS (Shivachandra *et al*.,

2005). An inactivation recombinant vaccine which expresses the fimbrial protein *P. multocida* B: 2 was found to provide significant protection and enhanced the stimulation of local and systemic immunities in goat when vaccination was given via intranasal against HS (Yasin *et al*., 2011).

This study was aimed to express a protein from ABA392/pET30a and focused on the immune response of the expressed protein for vaccine production against HS. The ABA392/pET30a recombinant plasmid was successfully constructed by ligating ABA392 gene into pET30a expression system and transformed into *E. coli* BL21 (DE3) pLysS bacterial strain. 6x His-tagged recombinant protein from ABA392-pET30a was successfully expressed due to tagging ABA392 gene in N-Terminal of pET-30a vector. The expressed protein was then purified using cobalt-based Immobilized Metal Affinity Chromatography (IMAC) system. No shock or other inflammations were observed in the vaccinated animals. The purified protein from ABA392-pET30a is immunogenic and has the capability to produce high titer antibody. This characterization shows that ABA392-pET30a might have a potential to be a candidate vaccine in this studies. Further characterization of the expressed protein and challenge studies using *P. multocida* serotype B: 2 against this protein are required to enhance the efficacy of this protein as a vaccine against HS in future.

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