J. Trop. Resour. Sustain. Sci. 11 (2023): 30-33
https://doi.org/10.47253/jtrss.v11i2.1238

Journal of Tropical Resources and Sustainable Science

journal homepage: jtrss.org

Back passage of haemorrhagic septicaemia (HS) vaccine seed C82 in cattle calf

Nurulaini Raimy[1,] *, Ho Hung Wui[1], Abdul Sukor Shaharudin[1], Rohayu Mohd. Nasir[1], Lily Rozita Md. Hakin[1], Norliza Wahab[1] and Rohaiza Yahaya[1]

[1]Veterinary Research Institute (VRI), Department of Veterinary Services, No. 59, Jalan Sultan Azlan Shah, 31400, Ipoh, Perak, Malaysia

Received 14 June 2023
Accepted 6 August 2023
Online 31 December 2023

Keywords:
Haemorrhagic septicaemia, *Pasteurella multocida*, vaccine, VRI

⊠*Corresponding author:
Nurulaini Raimy
Veterinary Research Institute (VRI), Department of Veterinary Services, No. 59, Jalan Sultan Azlan Shah, 31400, Ipoh, Perak, Malaysia
Email: nurulaini@dvs.gov.my

Abstract

Haemorrhagic septicaemia (HS) is caused by *Pasteurella multocida* serotypes B: 2, a Gram-negative coccobacilli bacterium. It causes fatal septicaemia with high mortality in cattle and buffaloes. Disease control and prevention are by effective HS vaccination in animals. A study was conducted on one mixed breed cattle calf for back passage of haemorrhagic septicaemia (HS) seed strain C82. Prior to bacterial inoculation in the calf, Mouse Protection Test (MPT) was conducted to ensure the calf is free from any HS antibody. The calf was injected subcutaneously with 0.5 ml dilutions of 10^{-3} of the bacteria incubated overnight (approximately 10^6 CFU/ml). After ±30 hours inoculation, post-mortem was conducted on the calf showing sign of HS. Heart blood and organ samples were collected and cultured on blood agar to determine bacteria purity. Blood and organ samples were also sent to the bacteriology laboratory in VRI for confirmatory tests. The death of the calf is confirmed by re-isolation of *Pasteurella multocida* Type B. Heart blood collected was processed, freeze-dried and keep as vaccine seed for further usage in vaccine production. Revival of vaccine seed is needed to ensure its pathogenicity and functionality for production purposes.

1. INTRODUCTION

Haemorrhagic septicaemia (HS) is an acute, highly fatal form of pasteurellosis caused by certain serotypes of *Pasteurella multocida* a gram-negative coccobacillus bacterium. The Asian serotype B:2 and the African serotype E:2 (Carter and Heddleston system), corresponding to 6:B and 6:E (Namioka-Carter system), are mainly responsible for the disease (OIE, 2021) It is one of the World Organisation for Animal Health (OIE) listed animal diseases and is considered the most economically important bacterial disease of water buffalo and cattle in tropical areas of Asia. It may cause highly fatal septicaemia (blood poisoning) with high morbidity and mortality and infects mostly cattle and buffaloes (Bain et al., 1982; Carter & De Alwis, 1989; De Alwis, 1992; Singh et al., 1996). A study by Habib et al. (2019) revealed that the mortality, morbidity and case fatality rates due to HS were greater in young calves than the adults both in buffaloes and cattle.

The geographical distribution of HS includes some areas of Asia, Africa, the Middle East and southern Europe. It has never been confirmed in Mexico, Central or South America (OIE, 2021). HS was first reported as early as the 1880s in Malaysia (FAO, 1991). In 2017, it caused a loss of almost RM1.8 million due to the death of 298 cattle and 56 buffaloes in Terengganu (Bernama, 2017; Harian Metro, 2017). Multiple HS outbreaks have been reported in Malaysia from 2017 to 2020, particularly in Terengganu. Malaysia has a population of cattle and buffaloes of 659,317 heads and 100,242 heads in 2020 which contributed to RM 1,595 million to our country (DVS, 2021). The most common and easy practice against HS is through the vaccination of animals (Zamri-Saad, 2013; Zamri-Saad and Annas, 2016). Veterinary Research Institute (VRI) under the Department of Veterinary Services (DVS) has been producing HS vaccine since 1949 (Lancaster, 1949). VRI started producing HS broth vaccine in 1949, HS Alum Precipitated (HSAP) in 1960, and HS Double Adjuvant (HSDA) in 1967 (Mustaffa-Babjee, 1994). For the past 10 years, a total of 514,725 doses of inactivated HS vaccines compromising of 318,550 double adjuvant and 196, 178 alum were produced. These vaccines were provided at no cost for Department of Veterinary Services (DVS) disease control and prevention activities and sold at a minimum price for private practitioners. HSAP and HSDA vaccine production using the *Pasteurella multocid*a Type B strain C82 which originated from a local disease outbreak as vaccine seed (Thomas, 1968). Therefore, reviving the *Pasteurella multocida* Type B seed strain C82 is crucial to maintain the viability and pathogenicity of the bacteria. In this study, one mixed breed, male cattle calf was used for back passage of *Pasteurella multocida* Type B vaccine seed strain C82. Prior to bacterial inoculation in the calf, Passive Mouse

eISSN Number: 2462-2389

Protection Test (PMPT) was conducted to ensure the calf is free from any HS antibody. HS vaccine seed strain C82 was inoculated in the host as recommended by Gowrakkal et al. (2014) and Dagleish et al. (2007).

2. MATERIALS AND METHODS

2.1 Experimental design

A study was conducted on one mix breed, male cattle calf for back passage of *Pasteurella multocida* Type B (PMB) seed strain C82. Prior to bacterial inoculation in calf, Passive Mouse Protection Test (PMPT) was conducted to ensure calf is free from any HS antibody. Mice were used as recommended by Bain et al. (1982) and Chandrasekaran et al. (1994). HS vaccine seed strain C82 was inoculated in host as recommended by Gowrakkal et al. (2014) and Dagleish et al. (2007).

2.2. Ethical approval

All animal handling procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of VRI with the animal ethics reference number IACUC/VRI (04/2019). Access to veterinary care was available at all times and the well-being of the animals used was assessed regularly.

2.3. Selection of cattle calf

Blood samples in EDTA and plain tubes were collected from sixteen cattle calves on a local government farm in Terengganu. Blood in plain tubes was centrifuged at 3000 x g for 15 minutes and processed for sera collection. Sera samples were also sent for melioidosis, brucellosis, Johne's disease, infectious bovine rhinotracheitis (IBR), bovine viral diarrhoea (BVD), bacterial isolation, virus isolation and Complete Blood Count (CBC) evaluation. All the sera samples were subjected to PMPT for evaluating immunity against HS vaccine in vaccinated or unvaccinated animals as described by Bain et al. (1982). Calves that are free from HS antibody were selected for back passage of HS seed strain C82. Calves are free from other bacterial disease that may interfere with the study. Selected animals were quarantined in VRI prior to inoculation and health screening was conducted.

2.4. Preparation of *Pasteurella multocida* Type B (strain C82)

A local strain of *Pasteurella multocida* C82 Carters type B was used in this study. First, the lyophilised seed was cultured on blood agar (BA - Oxoid; Cambridge, United Kingdom) supplemented with 5% cattle blood. An enriched liquid medium named Haemorrhagic Septicaemia Broth (HSB) which consists of mixture A (1% bacteriological peptone, 1% beef extract, 0.5% sodium chloride and 0.5% sodium bicarbonate in wt/vol) and mixture B (0.2% glucose, 0.4% yeast and 0.2% casein hydrolysate in wt/vol) was also prepared. The colonies in BA were evaluated according to their morphology and subjected to Gram staining to visualize the bipolar bacterial cells. Isolated colonies from BA were reconstituted into 200 mL of HSB and incubated at 37°C for 24 h.

2.5. Passive Mouse protection Test (PMPT)

PMPT was carried out according to Bain et al. (1982) and Chandrasekaran et al. (1994) for evaluation of protective effects in the vaccinated calves. The used of PMPT as an indicator of protective status against HS was also described by Reddy and Srinivasan (1998) and Tabatabaei et al. (2007). Briefly, a group of five Swiss albino mice aged 6-7 weeks were injected subcutaneously with 0.5 ml of neat calf's serum and challenged with 100-200 CFU of *Pasteurella multocida* Type B broth after 24 hours. An equal number of untreated mice were included as a control. These mice were obtained from the VRI Animal House and maintained in the experimental room according to the VRI Institutional Animal Care Usage Committee (IACUC). Mortalities were recorded and the experiment terminated after 7 days of observation. Throughout the study, all dead animals were subjected to post-mortem examination. The organ samples were cut, transferred into a sterile Petri dish (47 cm x 33 cm x 47 cm) and processed on the same day for bacterial isolation at Bacteriology Laboratory, VRI. The results are expressed as the percentage surviving out of 5 after a challenge with bacteria.

2.6. Passage of haemorrhagic septicaemia (HS) vaccine seed strain C82 in cattle calf

One cattle calf of 7-8 months old, clinically healthy which is free towards HS antibody based on PMPT conducted previously was selected for back passage of *Pasteurella multocida* Type B seed strain C82 to revive its pathogenicity. The calf was injected subcutaneously with 0.5 ml dilutions of 10-3 of the bacteria (HS seed strain C82) incubated overnight (approximately 106 CFU/ml). After 28 hours post inoculation calf showed clinical signs of HS i.e. difficulty of breathing, nasal discharge and collapsed (unable to stand). Post-mortem was conducted on the calf at about 30 hours post-inoculation. Organ samples (lung, heart, kidney, liver and spleen) were aseptically collected, put on a sterile Petri dish (47 cm x 33 cm x 47 cm) and cultured at the Bacterial Vaccine laboratory for isolation of *Pasteurella multocida* and sent for histopathology examination. Blood was collected aseptically from the heart and processed in a laboratory into small aliquots for long-term storage. The carcass was incinerated accordingly.

3. RESULT AND DISCUSSION

In PMPT calf serum inoculated in mice was challenged with diluted *Pasteurella multocida* Type B strain C82 bacterial broth. Two sera from cattle calves showed no protective effect (sero-negative) against *Pasteurella multocida* Type B in mice inoculated while the

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other fourteen cattle sera showed a 100% mouse survival rate and the control group showed 100% mortality. *Pasteurella multocida* Carter's Type B was isolated in abundance from the mice organ samples. PMPT is used to differentiate vaccinated and non-vaccinated ruminants or who have acquired natural immunity against HS.

In this study, only one cattle calf was used for the back passage of vaccine seed C82. The other calf was not included during the experimental infection of *Pasteurella multocida* Type B. Post-mortem examination of all vital organs including the lung, heart, kidney, liver and spleen appeared to be hyperaemic, congested and haemorrhagic. The gastrointestinal tract such as oesophagus, abomasum, duodenum, jejunum, ileum, caecum and rectum also showed similar findings. Straw-coloured blood tinge fluid was found in the thoracic region. Histopathological examinations of organ samples showed moderate to severe haemorrhage and congestion; necrosis and degeneration; and inflammatory cell infiltration in all organs. Also, lung showed mild to moderate oedema lesions.

Profuse growth of *Pasteurella multocida* Carter's Type B was found in heart blood and organ samples (lung, heart, kidney, liver and spleen) which indicated the septicaemia stage in animals causing death. *Pasteurella multocida* was confirmed by standard biochemical test and multiplex PCR (mPCR) by bacteriology Section VRI. Heart blood collected aseptically was stored as lyophilised cultures for further use in a -20 °C freezer. The *Pasteurella multocida* Type B strain C82 seed is being used in VRI for the past 20 years for HSAP and HSDA vaccine production. One recorded work of *Pasteurella multocida* vaccine seed passage was in 1965 via intranasal route into five calves (Chong, 1965). Four calves died and one calf was re-passaged via a subcutaneous route with 1.0 ml bacterial broth after the initial inoculation failed.

Calf selection in this study was based on PMPT followed by back passage of the C82 vaccine seed in cattle calves. PMPT is a recommended tool to evaluate the protective effect of the HS vaccine in animals (Bain et al., 1982; Chandrasekaran et al., 1994). It is used to differentiate vaccinated and non-vaccinated ruminants or who have acquired natural immunity against HS. A study by Phaswana et al. (2017) demonstrated the use of mice passive protection test to evaluate the humoral response in goats vaccinated with the Sterne 34F2 live spore vaccine. Mouse protection test was also used in suckling mice for the quantification of protective anti-foot-and-mouth disease virus (FMDV) antibodies (Mulcahy et al., 1991).

In our study, only two out of the sixteen calves were not having antibodies against HS. Therefore, one out of the two calves was chosen for back passage of *Pasteurella multocida* Type B strain C82 seed. Seropositive calf against HS could be due to maternal or active vaccination which we were unable to differentiate via PMPT.

Calf infected with the *Pasteurella multocida* Type B strain C82 culture showed respiratory distress, frothing from the mouth, nasal discharge, and subsequently recumbency and death 28 hours post-inoculation. The main characteristic feature of HS disease is septicaemia in all forms (OIE, 2021). At post-mortem there was also sero-sanguinolent fluid in the thoracic, pericardial and abdominal cavities with congested lungs. Although the incubation period of HS varies from 3 to 5 days, sudden death without clinical signs may be observed in peracute cases (OIE, 2021). Calf mortality due to HS showed that the vaccine seed C82 is still potent and able to cause HS experimentally.

Previous studies by Gowrakkal et al. (2014) reported that the immune efficiency of vaccine strain decreased when it is passage repeatedly in laboratory conditions (in vitro) therefore, it is important to conduct back passage in a natural hosts for longer immunopotency of the seed. Vaccine seed that was back passaged in its natural host had better immune efficiency to the culture than laboratory stock culture and it is recommended to conduct annual back passage of the vaccine seed culture of *Pasteurella multocida* bacteria for better establishment of immune potent vaccines (Gowrakkal et al., 2014).

Heart blood collected from the calf was processed and kept in lyophilised forms in 1 ml aliquots. For each new batch of the vaccine, an aliquot was thawed and cultured on a BA agar plate. Each seed used for vaccine production purposes will be tested accordingly to purity, safety and efficacy as recommended by OIE and ASEAN Standards. OIE recommended that seed cultures maintained as semisolid nutrient agar stab cultures at room temperature, or as lyophilised cultures.

Current findings indicated that back passage is useful to maintain the pathogenicity as well as to make sure vaccines produced by VRI using the C82 strain are working well. Seed strains may lose their potential or decrease immunopotential due to repeated passaging in unnatural hosts and stressful laboratory conditions.

Currently, VRI used *Pasteurella multocida* Type B strain C82 culture for the production of HS Alum Precipitated (HSAP) and HS Double Adjuvant (HSDA). To date, VRI is still producing around 60,000 doses of HS vaccines annually for the local ruminant industry, particularly DVS. In the future, we aim to improve vaccine formulation in terms of viscosity or better vaccine efficacy and delivery in livestock.

4. CONCLUSION

Pasteurella multocida Type B strain C82 culture was successfully revived for further usage in vaccine production.

eISSN Number: 2462-2389

ACKNOWLEDGEMENTS

The authors would like to thank the Director General of the Department of Veterinary Services Malaysia and the Director of Veterinary Research Institute (VRI), Ipoh for permission to publish the work and also all staff involved in this study.

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eISSN Number: 2462-2389