Short Communications

Immunogenicity of pasteurella multocida cell associated and cell free antigens in mice

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ABSTRACT

Haemorrhagic septicaemia is an economically important contagious disease of cattle, buffaloes and bison. In the present study, the efficacy of the vaccines prepared from cell free and cell associated antigens of disease causing organism *Pasteurella multocida* was evaluated in mice. From the present study, it was observed that both cell associated and cell free antigens provided protective immunity to the vaccinated animals in combination with each other and increasing the content of both the antigens improved the efficacy of the vaccine blends. In conclusion, use of both cell associated and cell free antigens in vaccine formulations are warranted for developing improved vaccines against Haemorrhagic septicaemia.

Key words: Haemorrhagic septicaemia, *Pasteurella multocida,* Cattle, Vaccines, Cell free antigen, Cell associated antigen.

Haemorrhagic septicaemia (HS) is an important fatal and contagious disease of cattle and buffaloes. It is caused by *Pasteurella multocida*, a Gram negative bipolar bacterium. The organism causes pathological changes in the respiratory tract leading to state of generalized septicaemic condition. The affected animal often leads to death in absence of timely treatment with antimicrobials. Due to its contagious nature, the disease can lead to economic devastation in the lives of farmers. In some parts of India alone, the disease is estimated to have caused losses to the tune of USD 1,30,000 between 2007 and 2011 (Singh *et al*., 2014).

Vaccination of susceptible hosts has been the most successful strategy for control of haemorrhagic septicaemia outbreaks. Inactivated vaccines are most commonly used for the purpose and include cell associated and cell free bacterial antigens along with one of the commonly used adjuvants such as alum, aluminium hydroxide and mineral oil. The oil adjuvanted vaccine provides high degree and duration of immunity compared to other adjuvanted vaccines. However, it suffers from the disadvantage of having high viscosity and poor syringibility

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during administration in the field (Saad and Anna, 2016). Therefore, present study was undertaken to evaluate the usefulness of aluminium hydroxide adjuvant with cell associated antigen and cell free antigens of *Pasteurella multocida.*

The *Pasteurella multocida* (P-52) vaccine strain procured from Indian Veterinary Research Institute, Izatnagar was passaged in healthy rabbit and the infected blood was stored in freeze dried form at 2-8°C. Further, the rabbit passaged freeze dried vaccine seed was used for preparation of vaccines with varying antigen payloads. Briefly, one vial of freeze dried rabbit passaged HS seed was inoculated into 200 ml of HS media and incubated at 37°C overnight under constant stirring. The 200 ml of HS vaccine strain seed culture was further inoculated into 10 L of HS media and incubated at 37°C overnight under stirring. The culture was inactivated by adding formalin to final concentration of 0.5% and incubating under stirring at 37°C for 24 h. The inactivated harvest was centrifuged at 6000 rpm for 45 min and, the supernatant and pellet were separated. The cell pellet was resuspended in 200 ml of 0.2% formal saline and again centrifuged at 6000 rpm and for 45 min. Subsequently, the cell pellet was resuspended in 180 ml of 0.2% formal saline and stored at 2-8°C until used for vaccine preparation. The 9.5 litre culture supernatant was concentrated to 460 ml by using synthetic hemodialyzer (ELISIO™ -13M, NIPPRO). Around 80 ml of pellet and concentrated culture supernatant was stored separately as source of cell associated and cell free antigens of *P. multocida*, respectively, while preparing vaccine blends. A total of four vaccine blends containing aluminium hydroxide as adjuvant were prepared namely; Blend-I: 1 ml culture equivalent of both cell associated antigen and cell free antigen, Blend-II: 2 ml culture equivalent of both cell associated antigen and cell free antigen, Blend-III: 1 ml culture equivalent of cell free antigen and Blend-IV: 1 ml culture equivalent of cell associated antigen (Table). All the four vaccine blends were tested for potency in mice by challenge with virulent *P. multocida* as per Indian Pharmacopoeia (2018) with prior approval from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The lethal dose 50 (LD_{50}) for each of the vaccine blends including unvaccinated controls were calculated by using Reed and Muench (1938). The titre of virulent *P. multocida* as observed in group of control mice was $10^{7.5}$ LD_{50}. The Protective Index (PI) was calculated as below.

PI = LD_{50} in control mice ÷ LD_{50} in vaccinated mice

LD_{50} = Reciprocal of 50% endpoint dilution.

Log_{10} 50% end point dilution = Log_{10} of dilution showing a mortality next above 50% - (Proportionate distance x logarithm of dilution factor).

Proportionate distance = [(mortality at dilution next above 50%) – 50%] / [(mortality next above 50%) – (mortality next below 50%)]

Table 1 Composition of vaccine preparations with cell associated antigens and cell free antigens of *Pasteurella multocida*

Blend	Antigen type	Culture volume equivalent/ dose	Antigen volume/dose	Aluminium hydroxide / dose	0.2% formal saline / dose	Potency (Protection Index) Log_{10} values
I	Cell associated + Cell free	1 ml	0.1 ml	0.9 ml	1 ml	4.1
II	Cell associated + Cell free	2 ml	0.2 ml	0.9 ml	0.9 ml	4.5
III	Cell free	1 ml	0.1 ml	0.9 ml	1 ml	3.5
IV	Cell associated	1 ml	0.1 ml	0.9 ml	1 ml	3.7

The calculated PIs for Blend-I containing 1 ml culture equivalent of cell associated and cell free antigen /dose was 4.1, Blend-II containing 2 ml culture volume equivalent/dose containing both cell supernatant and cell pellet was 4.5, Blend-III containing 2 ml culture volume equivalent of cell supernatant was 3.5 and Blend-IV containing 1 ml culture volume equivalent/dose of cell associated antigen was 3.7. A close analysis of results reveal that the blends having combination of both cell associated and cell free antigen provided better protection to virulent *P. multocida* challenge in mice but no protection when administered alone. Increasing the antigen content of both the antigens (2 ml culture equivalent) yielded marginally better protection compared to 1 ml culture volume equivalent of antigens. Various factors such as outer membrane proteins (OMP), lipopolysaccharides, secreted bacterial toxins and stress induced proteins have been identified to play an important role in the pathogenesis and host cell evasion of *P. multocida* (Ghani *et al*., 2016). These factors also act as targets for host immune responses leading to development of protective immunity against the infecting organisms. Recently, Uchida *et al*., (2003) showed higher protective indices and prompt clearance of toxigenic strains of *P. multocida* in mice immunized with inactivated cell free antigen than purified and inactivated *P. multocida* toxin. Similarly, 100% protection in mice immunized with cell associated antigen (OMP) has also been reported (Joshi *et al.,* 2013). In conclusion, present study showed that both cell associated antigens (such as OMP and capsular antigens) and cell free antigens (toxins and stress proteins) elicit immune responses and the protective immunity is enhanced by combining both the antigens in the vaccine formulations.

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