Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2023.044

RESEARCH ARTICLE

Preparation, Safety and Efficacy of Live Aerosol Hemorrhagic Septicemia Vaccine in Buffaloes and Cattle

Sajid Mahmood Sajid[1,3], Arfan Yousaf[1]*, Hamid Irshad[2], Muhammad Arif Zafar[1] and Saif ur Rehman[1]

[1]Faculty of Veterinary and Animal Sciences, PMAS-Arid Agriculture University, Rawalpindi
[2]Animal Health Program, Animal Sciences Institute, National Agricultural Research Center, Islamabad
[3]Faculty of Veterinary and Animal Sciences, University of Poonch Rawalakot, Azad Kashmir
*Corresponding author: arfanyousaf@uaar.edu.pk

ARTICLE HISTORY (23-095)

Received:	March 23, 2023
Revised:	June 04, 2023
Accepted:	June 09, 2023
Published online:	June 18, 2023

Key words:
Buffalo
Cattle
Intranasal vaccine
Pasteurella multocida
trehalose

ABSTRACT

Hemorrhagic septicemia (HS) is an acute and highly contagious disease of water buffaloes and cattle. Financial losses due to HS made it an important disease. HS control is generally attained with subcutaneously injected killed vaccines. However, with some other de-merits, these vaccines provide only short-term protection and are difficult to administer. In present study, live aerosol vaccine was prepared using live *Pasteurella multocida* B:3,4 that was non-pathogenic strain and tested for efficacy in buffaloes and cattle. The live aerosol vaccine was prepared using 15% trehalose as stabilizer. Safety test of vaccine was conducted in calves. Each calf was administered 100 times the recommended dose of vaccine through intra-nasal route. The calves were kept under observation for 14 days. Vaccinated animals did not show any undesirable reactions. Body temperature remained normal. Food and water intake were as usual. Results suggestively exhibited the safety of the vaccine. For efficacy test, buffaloes and cattle were divided into two groups. The animals of one group were given live aerosol HS experimental vaccine at the dose of 1 ml/animal containing 2.7×10^7 viable organisms through intra-nasal route. The animals of second group were injected with commercially available killed HS vaccine at the dose of 3 ml/animal through subcutaneous route. Protective anti-LPS-IHA antibody titer against *P. multocida* for live aerosol vaccine was detectable up to 12-month post vaccination while killed vaccine titer was detectable up to 6 months. Results revealed that the antibody levels in animals vaccinated with aerosol vaccine were significantly higher than killed vaccine. The efficacy of the vaccine was further evaluated using challenge protection test. Two groups of animals containing vaccinated and unvaccinated animals were challenged with 1 ml of the diluted broth culture containing 2×10^7 viable organism of *P. multocida* B:2 that was pathogenic strain. Observation of both the groups for 14 days revealed that unvaccinated animals displayed signs of HS disease while animals of vaccinated group protected against the challenge. Results of challenge protection test confirmed the efficacy of live aerosol HS vaccine. It was concluded that the live aerosol vaccine conferred improved protective immunity against *P. multocida* B:2 infection and reduced frequency of the vaccination in buffaloes and cattle.

To Cite This Article: Sajid SM, Yousaf A, Irshad H, Zafar MA and Rehman SU, 2023. Preparation, safety and efficacy of live aerosol hemorrhagic septicemia vaccine in buffaloes and cattle. Pak Vet J, 43(3): 449-455. http://dx.doi.org/10.29261/pakvetj/2023.044

INTRODUCTION

Hemorrhagic Septicemia is one of the production limitation bacterial diseases of livestock in Pakistan and other developing countries in Asian region (Farooq *et al*., 2007). The etiological agent responsible for the disease are *Pasteurella multocida* (*P. multocida*) serotype B:2 and E:2. Categorization of the agent is based on capsular antigen and designated as A, B, D, E and F and on lipopolysaccharide antigen adopting Heddleston system (group 1-16) (Carter, 1955; Heddleston *et al*., 1972). The strain B:2 is Asian strain and E:2 is African strain (Carter, 1955; Dawkins *et al*., 1990; Moustafa *et al*., 2015). The disease affects the large and wild ruminants. The disease intensity is severe in buffaloes compared to cattle. However, older animals are less susceptible than young

and young adult animals (De-Alwis, 1992; Farooq *et al*., 2007; Shivachandra *et al*., 2011; Shome *et al*., 2019).

HS is economically significant disease (Benkirane and De-Alwis, 2002). The susceptible animals showed 34.1% mortality in Pakistan (Farooq *et al*., 2007; Tasneem *et al*., 2009). In Pakistan annual losses of PKR 2.17 billion have been estimated due to HS (Ashraf *et al*., 2011). The actual losses could be much higher than estimated losses as these may not include reduction of productivity, milk, meat, draught power, and cost of alternate sources of draught power and impairment of the reproductive potential of the animals (Benkirane and De-Alwis, 2002; Singhla *et al*., 2020).

Acute nature of disease makes it difficult to treat animals because they remain unnoticed and result in immediate death (De-Alwis, 1992; Ahmad *et al*., 2014; Gulia and Aly, 2020). Recovery of animals with HS is possible if treated in early stages of the disease, which is usually not possible in primitive husbandry systems (Singh and Manoj, 2018; Singh *et al*., 2019). Moreover, treatment of HS requires prolonged antibiotic treatment which could be expensive and may lead to emergence of antimicrobial resistance (Ahmad *et al*., 2014; Cuevas *et al*., 2020). The only possible solution to this is husbandry measures and prophylactic vaccination, which are routinely used.

The definitive purpose of vaccine is to protect animals from various diseases. Some diseases including HS are well controlled using vaccines (Farooq *et al*., 2019). Various types of killed vaccines are available for the control of HS like aluminum hydroxide gel vaccine, broth bacterin, alum precipitated, multiple emulsion and oil adjuvant vaccine. Killed vaccines are extensively used against HS throughout the world (Shivachandra *et al*., 2011; Poudel *et al*., 2020). Homologous vaccine with virulent strains of *P. multocida* (B:2 or E:2) are being used to prepare killed vaccines for HS (Tasneem *et al*., 2009; Varshney *et al*., 2020). Nonetheless, these injectable vaccines are difficult to administer, as restraining the animals is a field problem (Mostaan *et al*., 2020). Bacterins does not possess the ability to produce cross serotype defense, results in ineffective and protection for short period of time (Gyles *et al*., 2004; Nassar *et al*., 2012; Chelliah *et al*., 2020). Killed vaccines may cause inflammatory reaction at administration site (Ahmad *et al*., 2014; Ghadimipour *et al*., 2021). Oil adjuvant vaccine is fairly disliked by farmers because it possesses dense viscosity that makes it difficult to inject (De-Alwis, 1992; Rahman *et al*., 2016). Moreover, abscesses, swelling and post vaccination shock with oil adjuvant vaccine have been observed (Zamri-Saad, and Annas, 2016). Alum precipitated vaccine is extensively used, but it produces weak immune response and is not stable (Shivachandra *et al*., 2011; Rita *et al*., 2018).

To overcome these problems, live aerosol vaccine could be the substitute. Intra-nasal route is easy and convenient for vaccine administration compared to injectable and could protect animals for longer duration (Wang *et al*., 2015). A live heterotypic vaccine containing serotype B:3,4 isolated from a fallow deer protected cattle against virulent B:2 challenge in Myanmar (Myint *et al*., 2005; Herliani *et al*., 2020). It conferred solid and long term protective immunity and cross protection against heterologous serotypes challenge.

However, stability and conservation of number of organisms as per recommended dose in live vaccine is an issue, and this is affected by variation in temperature at which vaccine is stored. Use of various stabilizers in vaccines may overcome this problem. In some studies, the effect of different types of stabilizers on viability at various storage temperatures was evaluated. As a result, 15% trehalose and -20°C were found most suitable stabilizer and storage temperature for heterotypic aerosol hemorrhagic septicemia vaccine (Oslan *et al*., 2017; Sajid *et al*., 2021).

In this study, we used 15% trehalose as stabilizer to prepare live heterotypic aerosol vaccine. Afterward, efficacy of live vaccine was evaluated in buffaloes and cattle. An oil based killed vaccine was also administered to other group of animals for comparison.

MATERIALS AND METHODS

Bacterial strains: Two strains of *P. multocida* were used in this study. The vaccine strain was *P. multocida* B:3,4 which was used to prepare live aerosol hemorrhagic septicemia vaccine (Myint *et al*., 2005; Saleem *et al*., 2014). The strain for challenge protection test was *P. multocida* B:2 (Myint *et al*., 2005; Kamal *et al*., 2017).

Source of bacteria: Isolates of *P. multocida* serotype B:3,4 and B:2 were recovered from the inventory at -20°C maintained at Bacteriology Lab, Animal Health Program, Animal Sciences Institute, National Agricultural Research Center (NARC), Islamabad.

Revival of Bacterial Strains: Mice inoculation method was used to revive *P. multocida* B:3,4 and B:2 strains. Method adopted according to Singh *et al*. (2010) with minor modifications. Albino mice (n=6) were purchased from the National Institute of Health (NIH), Islamabad. The mice were kept and reared in Laboratory Animal House, Animal Health Program, NARC. These mice were divided into two groups, Group-I (n=3) and Group-II (n=3). Lyophilized *P. multocida* B:3,4 strain was reconstituted in 1 ml normal saline (pH=7.2±0.2) and inoculated onto tryptic soya agar (TSA) plates. The plates were incubated at 37°C for 24 hours. After incubation, mice in Group-I were injected intraperitoneal with 200 µL of 10^{-5} dilution *P. multocida* B:3,4 culture. The mice in Group-II (control group) were injected with 200 µL of normal saline solution. After 24 hours, mice inoculated with *P. multocida* B:3,4 died. However, the mice of control group remained alive. Spleen and heart were collected aseptically from dead mice after dissection and used to streak on blood agar. The heart blood was also collected and streaked on blood agar plates. These streaked plates were incubated for 24 hours at 37°C. Similar protocol was adopted to revive *P. multocida* B:2 strain for challenge protection studies.

Preparation of Vaccine Suspension: Pure culture of *P. multocida* B:3,4 was streaked on TSA plates for bulk growth and plates were incubated at 37°C for 24 hours. After 24 hours the growth was harvested in Tryptic Soya Broth (TSB) for enrichment and incubated at 37°C for 24

hours. In resulting bacterial suspension, number of organisms was adjusted using Miles and Misra method (Miles *et al*., 1938) Reference). Trehalose solution (15%) was used as stabilizer for vaccine preparation (Oslan *et al*., 2017; Sajid *et al*., 2021). Bacterial suspension and stabilizer are mixed in 1:1 ratio for vaccine preparation (Dumpa *et al*., 2019). Vaccine was lyophilized and stored at -20ºC.

Indirect haemagglutination test for *P. multocida* antibodies: Indirect haemagglutination (IHA) test was applied to detect antibodies against *P. multocida* (Das *et al*., 1998; Tankaew *et al*., 2018).

Preparation of antigen for IHA: Casein Sucrose Yeast (CSY) agar was used to culture B:2 strain of *P. multocida*. A 6-8 hours' culture of *P. multocida* B:2 was cultured on CSY agar and incubated at 37°C for 24 hours. Physiological saline (3 ml) containing 0.3% formalin was used for harvesting the growth. Afterward, this suspension was heated at 56ºC for half an hour and centrifuged at 3000 *g* for 15 minutes at 4ºC. A clear supernatant liquid was used as antigen extract and stored at -20ºC (El-Jakee *et al*., 2016).

Sensitization of erythrocytes: Chicken erythrocytes were sensitized with antigen as per method described by Fraser *et al*. (1983). In short, 200 µL of washed RBCs suspension was mixed with 3 ml of bacterial antigen and incubated at 37°C for 1 hour with frequent shaking in shaker incubator. Sensitized erythrocytes were recovered by centrifugation and then erythrocytes were washed three times with normal saline and finally 1% suspension was prepared in physiological saline.

IHA Procedure: The IHA test was performed according to Das *et al*. (1998). The test was performed in 'U' shape micro-titer plate. In summary, 50 µL of diluent (normal saline) was dispensed in all wells of micro-titration plate. The 50 µL of serum was poured in first well of the row and serially diluted up to well number 11 i.e., serum sample was added in first row. From this row 50 µL was taken with pipette and poured in next well and after mixing 50 µL was taken and poured into next well. This was done up to well number 11. The sensitized RBCs suspension (50 µL) was added in all wells of micro-titration plate. Plate was then stored at 37°C for 1 hour. Results were noted after 1 hour.

Safety testing in calves: Safety testing procedure was adopted according to the Shah *et al*. (2001). Safety of live aerosol vaccine was tested on cattle and buffalo calves of aging between 6-8 months. A total of 4 calves, were selected from Livestock Research Station (LRS), NARC. Calves were selected on the basis of absence of antibodies against *P. multocida*. The antibodies were determined using IHA. All calves were kept and managed at Animal House, NARC. Each calf was given 2×10^9 CFU/ml viable organisms (100 times the recommended dose) of *P. multocida* B:3,4 through intra-nasal route. The calves were kept under observation for 14 days' post vaccination. Rectal temperature of animals was recorded on daily basis to assess febrile conditions, if any. Blood samples were collected at the end of experiment. IHA was performed on extracted serum to estimate the titer of antibodies.

Efficacy testing in cattle: Efficacy trials were initiated at, Livestock Development Research Center (LDRC), Muzaffarabad and Dairy Farms, Quetta. Cattle (n=66) were selected at LDRC while buffaloes (n=64) were selected at Dairy Farms Quetta. Serum samples from these animals were taken to evaluate the presence of antibodies against HS vaccine using IHA. All the animals had no antibodies against HS vaccine. These were divided into two groups. The buffaloes (n=32) and cattle (n=32) in Group 1 were given live HS experimental vaccine at the dose of 1 ml/animal containing approximately 2.7×10^7 viable organisms through intra-nasal route. A simple spray bottle as aerosol sprayer was used for intra nasal vaccination. Sprayer was filled with live aerosol vaccine and tip of the sprayer was brought near to the nostril of animal and sprayed quickly to make sure that vaccine has entered upper respiratory tract of the animal. The buffaloes (n=32) and cattle (n=34) in Group 2 were given commercially available killed HS vaccine at the dose of 3 ml/animal through subcutaneous route according to the manufacturer's instruction. The serum samples were collected from experimental animals on monthly basis for first 6 months then every second month up to 12 months and analyzed using IHA to determine the presence of antibodies against HS vaccine.

Challenge protection test: The efficacy of the vaccine was further evaluated using challenge protection test as described by Myint *et al*. (2005). A total of 8 apparently healthy animals were selected divided into two groups (Table 2). Group A (n=4, control, unvaccinated) and group B (n=4, vaccinated). The animals of group B were selected from the animals vaccinated with live aerosol vaccine for trial. The animals of group A and B were inoculated subcutaneously with 1 ml of the diluted broth culture containing 2×10^7 viable organism of *P. multocida* B:2. The animals of both groups were observed for 2 weeks for development of clinical signs. Animals were monitored for the overall behavior (normal, dull, depressed, or recumbent) at intervals of 4 h for 2 weeks in order to characterize clinical signs to challenge or immunization (Tabatabaei *et al*., 2007). Nasal swabs were collected before and after inoculation of *P. multocida* B:2 on daily basis. The swabs were cultured on blood agar plates, incubated overnight at 37°C. The isolates were confirmed as *P. multocida* B:2. Postmortem was performed in dead calf and gross lesions were recorded. Tissues and organs obtained at necropsy were also cultured for bacteria on blood agar and confirmed as *P. multocida* B:2.

Statistical analysis: Geometric mean titer (GMT) values of IHA antibody titers were calculated as by Villegas and Purchase (1989) and Burgh, (1998) to compare the efficacy of vaccines.

RESULTS

Safety test in cattle and buffalo calves: Calves administered with 100 times the recommended dose of vaccine did not show any undesirable reactions. Body temperature did not show significant variation at all. Food

and water intake remained normal. There was no irregularity or deviation in demeanor. Other clinical signs of disease did not appear in calves during observation period.

Effect of vaccines on antibody titer of cattle: Results of live vaccine (Fig. 1) indicated that protective level of anti-LPS-IHA antibody titer against *P. multocida* appeared at day 60 post vaccination and GMT±S.D was 4.4±0.50, while killed vaccine showed protective level at day 90 and GMT±S.D was 4.5±0.5 (Fig. 1). The maximum level of titer with live vaccine was at day 90 (5.6±0.48), however, killed vaccine showed peak titer at day 120 (5.0±0.55). Up to day 360, serum samples of animals administered with live aerosol vaccine showed protective level of titer (4.1±73) against *P. multocida.* On the other hand, protective level of titer with killed vaccine was observed up to day 180 (4.0±1.34) then a decline in titer level was observed below the protective level. CGMT±S.D with live vaccine was 4.5±1.34 (Table 1) while CGMT±S.D for killed vaccine was calculated as 3.0±1.64 (Table 1). Overall, results revealed that the antibody levels in animals vaccinated with aerosol vaccine were significantly higher than killed vaccine.

Effect of vaccines on antibody titer of buffaloes: In buffaloes, monitoring anti-LPS-IHA antibodies with live HS vaccine (Fig. 2) showed that the titer was initiated to increase effective level on day 60. GMT and standard deviation (GMT±S.D) of titer at day 60 was 4.56±0.50. Antibody titer was maximum (5.72±0.45) at day 90. While titer was dropped on day 120 and exhibited GMT±S.D as 5.65±0.48. Results indicated that the titer with live vaccine remained protective up to day 360 showing GMT±S.D as 4.0±0.91. Cumulative geometric mean titer (CGMT±S.D) with live vaccine was calculated as 4.54±1.27 (Table 1). On the other hand, killed vaccine in buffaloes (Fig. 2) revealed that antibody titer was protective at day 90 and GMT±S.D was 4.69±0.48. The titer (5.52±0.51) was maximum at day 120. Results demonstrated that antibody titer was at effective level up to day 180 (4±0.39) then started to decline below protective level. While CGMT±S.D with killed vaccine was calculated as 3.24±1.56 (Table 1).

Effect of challenge with *P. multocida* B:2 strain: The results (Table 2) showed that no clinical signs or disruption in behavior was noted in the group of the vaccinated animals challenged 12 months' post vaccination. The food and water intake were also normal in the animals of this group. All animals survived (Table 2) and the percentage of protection was 100. Whereas, after challenged with pathogenic *P. multocida* strain B:2, all non-vaccinated control animals showed the clinical signs of HS within 48 hours of challenge. The clinical signs observed were dullness, reluctance to move, anorexia, high temperature (104.0-105.0°F), respiratory distress and salivation. Edema of the submandibular area was also detected. Morbid animals died. Postmortem was carried and samples were collected from different organs including lungs, liver, spleen and heart. These samples were used to streak on media and all the plates were incubated at 37°C for 24 hours. The challenge strain (*P. multocida* B:2) was recovered from these organs.

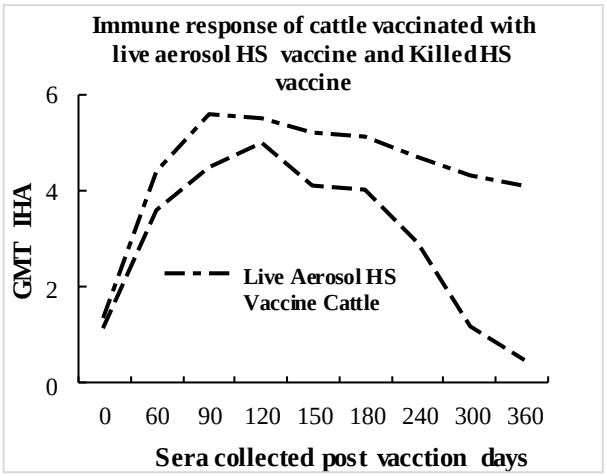

Fig. 1: Immune response of cattle vaccinated with live aerosol hemorrhagic septicemia (HS) vaccine and killed HS vaccine.

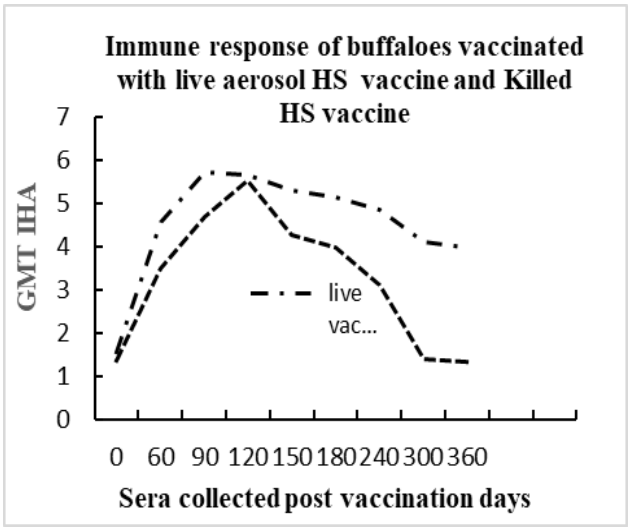

Fig 2: Immune response of buffaloes vaccinated with live aerosol HS vaccine and Killed HS vaccine.

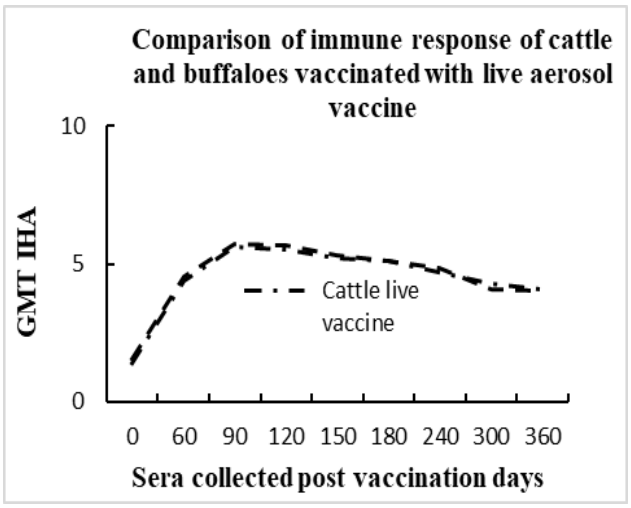

Fig 3: Comparison of immune response of cattle and buffaloes vaccinated with live HS aerosol vaccine.

Table 1: Immune response (GMT±S.D) of buffaloes and cattle vaccinated with live and killed vaccines

Vaccine	Animal type	Post vaccination days									
		0	60	90	120	150	180	240	300	360	CGMT±S.D
Live	Cattle	1.4±0.49	4.4±0.50	5.6±0.48	5.5±0.51	5.2±0.45	5.1±0.38	4.7±0.46	4.3±0.48	4.1±0.73	4.5±1.34
	Buffaloes	1.57±0.59	4.56±0.50	5.72±0.45	5.65±0.48	5.31±0.47	5.13±0.35	4.87±0.34	4.1±0.65	4±0.91	4.54±1.27
Killed	Cattle	1.2±0.46	3.6±0,50	4.5±0.51	5±0.55	4.1±1.42	4±1.34	2.9±1.43	1.2±0.43	0.5±0.51	3.0±1.64
	Buffaloes	1.38±0.49	3.5±0.51	4.69±0.48	5.52±0.51	4.26±1.16	4±0.39	3.1±0.91	1.4±0.4	1.33±0.51	3.24±1.56

Table 2: Percentage protection of calves with virulent *P. multocida* B:2 challenge applied 12-month post vaccination.

Groups	No. of animals challenged	Morbidity	Observations post challenge	Conclusion
A (Control)	4	4	All morbid	0% protection
B (Vaccinated)	4	0	All survived	100% protection

DISCUSSION

Live aerosol vaccine used in this study was prepared in 15% trehalose as stabilizer. This formulation proved to be stable in terms of viability of *P. multocida* B:3,4 vaccine strain (Sajid *et al*., 2021). We used *P. multocida* B:3,4 as vaccine strain because it is nonpathogenic and is not considered the cause of HS (Dawkins *et al*., 1990; Jones, 2018). Secondly, this strain contains capsular antigen B that is capable of inducing immunity in vaccinated animals (Myint *et al*., 1987; Rimler *et al*., 1987).

A possible objection to use *P. multocida* B:3,4 as vaccine strain may introduce a probable pathogen into Pakistan or Asia. However, documentation of occurrence of this strain in variety of apparently healthy or sick animals explains that it has been undoubtedly distributed in whole world since many years (Aalbæk *et al*., 1999). In Myanmar, this strain has been used extensively for many years as live vaccine without generating any unwanted responses in the vaccinated or wild animals and birds in the locality (Jones *et al*., 1996).

In current study, the IHA test was used to detect antibodies against *P. multocida*. The findings of Priadi and Natalia (2002) and Myint *et al*. (2005) demonstrated that aerosol immunization with *P. multocida* B:3,4 generated systemic immunities that could be detected by IHA or challenge protection test. The IHA test has also been recommended for detection of the capsular antigen of *P. multocida* (Carter, 1955; Das *et al*., 1998; Kayani *et al*., 2000; Tankaew *et al*., 2018). Antibodies of the IgG isotype are generated against pathogenic microorganisms in humoral immune responses (Nimmerjahn, 2014; Sebina and Pepper, 2018). Value of $\log_2^4$ to $\log_2^6$ is considered protective range of IgG titer for HS vaccine (Myint *et al*., 2005; Sarwar *et al*., 2015).

Results from safety testing demonstrated that the heterotypic aerosol vaccine is innocuous even at high dose levels as well. It did not induce any anaphylactic shock or local unwanted reactions that are occurred with other HS vaccines administered by parenteral route. In our trial aerosol vaccine did not show any death in cattle. Therefore, aerosol vaccine was proved safe in cattle. This is in agreement with studies of Myint *et al*. (2005) and Saleem *et al*. (2014).

Our results indicated that protective level of titer with live aerosol vaccine raised as earlier (at day 60) than killed vaccine (at day 90). The reason is that live aerosol vaccine follows the natural route of infection of HS and more readily able to move to anatomical sites and started to produce IgG (Belyakov and Ahlers, 2009). Moreover, aerosol route generally has the potential to stimulate a mucosal immune response, along with a humoral (Chase and Kaushik, 2019). Secretory IgA in mucosal immunity could neutralize infectious agents at the site of entry and eventually prevent infection. In addition, mucosal immunity can affectedly reduce local tissue spread, shedding and transmission to other animals (Palomares *et al*., 2021). Our results were supported by the findings of Makoschey (2015), which indicated that route of vaccination and methods of administration have a great influence on efficacy of vaccine.

Our results indicated that duration of protection was significantly different as it was greater with live vaccine i.e., up to 360 days. It is due to the reason that live vaccines stimulate both humoral (B cells) and cellular (CD8+and CD4+ T cells) (Kollmann, 2013; Furman and Davis, 2015; Ahmad *et al*., 2018; Almoheer *et al*., 2022). Moreover, live bacteria replicate for long time resulted enhanced immune response (Hajer and Hassan, 2018). The results are in agreement with the studies of Tabatabaei *et al*., (2007). Similar outcomes were also reported in the studies of Sarwar *et al*. (2015). Our findings were also supported by a previous study of Rafidah *et al*. (2011) using the same strain live vaccine significantly increased the overall animal herd immune response for a minimum of 10 months. The protective antibody levels of serum IgG responses with killed vaccine was observed up to day 180 post vaccination. This is due to the fact that the killed vaccines could not confer effective long-term defense owing to the possibility of destruction of the pathogen replication by host defense mechanism (Cho *et al.*, 2002; Jorge and Dellagostin, 2017).

This study proved that immunization requires a low dose of intranasal aerosol vaccine containing viable organisms 2×10^7 as compared to the live *Brucella abortus* strain RB51and strain 19 vaccines, which need at least 10^9 and 10^{10} viable organisms per dose (Dorneles *et al.*, 2015). Our study showed that vaccination with 2×10^7 live organisms of *P. multocida* B:3,4 could be administered safely through intranasal route to animals aging more than 6 months. This agrees with studies of Myint *et al.* (2005) and Saleem *et al.* (2014).

The current study also proved that immune response of buffaloes and cattle vaccinated with live vaccine showed alike immune response (Fig 3) and analogous rise in antibody titer. No significance difference was found in immune response of both types of animals. It proved that live HS vaccine is equally effective in both types of animals. Our findings are agreeable with the results of Tabatabaei *et al.* (2007) and Rafidah *et al.* (2011).

In our studies, a single dose of aerosol vaccine provided an extended immunity as compared to killed vaccine. A single dose of administered bacteria replicated

in the animals and produced enough of the organism to stimulate a prolong immune response whereas killed vaccine requires booster doses to attain similar protection. These results are agreeable with the study of Zimmermann and Curtis, (2019). Likewise, studies of Myint *et al*. (2005) and Natalia and Priadi (2005) on live HS vaccine using *P. multocida* B:3,4 exhibited that a single intranasal vaccination produced a high titer of antibodies that conferred defense to cattle for at least one year. The results of the challenge protection test verified that the vaccine was effective as 100 percent protection was observed in vaccinated animals challenged. The recovery of the challenge strain *P. multocida* B:2, in unvaccinated animals is also evidence of the effectiveness of the vaccine.

Conclusions: The present study provided the possibility of intranasal vaccination with a live aerosol preparation from *P. multocida* against HS in cattle and buffaloes with strong antibody response and protection levels. Live aerosol vaccine is convenient to administer to animals while a single dose provides strong immunity for one year. Single dose strategy would be economical for the farmers. The parenteral vaccines require asepsis as well. Whereas aerosol vaccine does not need asepsis that makes convenient to work with many animals. However, there is needed to develop the vaccine on a commercial scale so that farmers can take full advantage of this vaccine.

Acknowledgements: Authors thank Agricultural Linkages Program, PARC (Project No: AS-142) for financial assistance for this study. Authors also thank to the Dr. Muhammad Ijaz Khan, Director General, and whole staff of Livestock and Dairy Development Department, Muzaffarabad, AJK and Dairy Farms, Quetta for facilitating research trials.

Authors contribution: SMS: Planning of study, standardization and execution of lab protocols, collection and analysis of samples from experimental animals, analysis of data and write up of manuscript, AY: Planning of study, analysis of data and write up of manuscript, H I: Planning and execution of study, analysis of data and write up of manuscript, M AZ: Planning of study and analysis of data, SuR: Planning of study and write up of manuscript.

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