Indian Journal of Animal Sciences **93** (9): 853–857, September 2023/Article
https://doi.org/10.56093/ijans.v93i9.114913

Kinetics of humoral response to *Pasteurella multocida* in buffaloes against combined foot-and-mouth+haemorrhagic septicaemia vaccine

NEENA RUSTAGI[1], NARESH KUMAR KAKKER[1✉], SUMAN SHEORAN[2], PATIL CHANDRASHEKHAR SANTOSH[1], SWATI DAHIYA[1] and AKHIL KUMAR GUPTA[1]

Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana 125 001 India

Received: 5 September 2021; Accepted: 9 August 2023

ABSTRACT

The present study reports kinetics of anti-*P. multocida* antibody (Ab) response at monthly intervals in Murrah buffaloes of different age groups vaccinated against combined Foot-and-mouth disease+haemorrhagic septicaemia (FMD+HS) vaccine. A total of 60 Murrah buffaloes of three age groups having 20 animals each: calves, heifers and adults were used to monitor anti-*P. multocida* Ab response at monthly intervals using single dilution indirect ELISA. The percentage of adult buffaloes protected were found to be the highest during all the six months post-vaccination followed by heifers and calves. The protective mean Ab titres were maintained up to six months post-vaccination for heifers and adults but not for calves. The F value (the ratio of two mean squares) for pre- and all the six month(s) post-vaccination and all the three age groups was significantly higher. Pearson Chi square value for pre-vaccination and all the six months except three months post-vaccination was significantly higher. Pearson correlation value was significantly higher with positive linear relationship. The data in the present study indicated that the combined FMD+HS vaccine was found to be effective in buffaloes of all age groups at government organised farm and could be an ideal approach in field conditions under Livestock Health Disease Control Program run by the Government of India.

Keywords: Antibody titres, Combined FMD+HS vaccine, Haemorrhagic septicaemia, *Pasteurella multocida*, SdiELISA, Sero-monitoring

Haemorrhagic septicaemia (HS) is one of the most economically important bacterial diseases mainly of cattle and buffaloes (OIE 2021) caused by gram-negative coccobacillus *Pasteurella multocida* subspp. *multocida* belonging to the family Pasteurellaceae. HS is the second most reported disease in India during the last two decades, prevalent throughout the country and responsible for approximately 60% of bovine mortality (Pal *et al*. 2017) and is among the major health challenges facing the livestock industry in South Asian countries (Habib *et al*. 2019, Shome *et al*. 2019). It results in huge economic losses annually in the subcontinent (Muenthaisong e*t al*. 2021). The huge economic losses due to HS can be minimized by proper vaccination and sero-monitoring of vaccinated animals. Short duration immunity of only 4-6 months is a major limitation of the conventional killed bacterial vaccines.

Of late, the combined Foot-and-Mouth Disease (FMD)+HS+Black Quarter (BQ) and FMD+HS oil adjuvanted vaccines are also available commercially (Prasad *et al*. 2019) which have advantage of not only reducing the number of pricks being given to the animals but also have economical advantage in terms of cost of vaccine, logistics, manpower, maintenance of cold chain, etc. as compared to individual vaccines. Further, the farmers may also save a lot of time annually in getting their animals vaccinated with combined vaccines.

The monitoring of humoral immune response through detection of IgG antibodies (Abs) is usually done for monitoring the response of vaccination and development of immunity. ELISAs have proven efficacious in the quantification of the Ab as well as Ag with high sensitivity and specificity (Mahboob *et al*. 2023) and is suitable for screening of a large number of field samples simultaneously (Tankaew *et al*. 2018, Amanat *et al*. 2020).

The perusal of available literature shows that there is paucity of data on sero-monitoring of anti-*P.multocida* Abs in vaccinated buffaloes. The present study reports kinetics of anti-*P. multocida* antibody response at monthly intervals in Murrah buffaloes vaccinated against combined FMD+HS oil adjuvanted vaccine.

MATERIALS AND METHODS

Animals, vaccine and vaccination: A total of 60 Murrah buffaloes maintained at State Cattle Breeding

Present address: [1]Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana. [2]State Cattle Breeding Project-II, Hisar, Haryana. ✉Corresponding author email: nkmksk@rediffmail.com

Project, Hisar, Haryana (located at 29.1924°N latitude and 75.6933°E longitude) were used in the present study. The animals were divided into three age groups having 20 animals each: Group A (calves, <1 year); B (heifers, 1 to 3 years) and C (adults, >3 years) having been given 1-2, 3-5 and >5 FMD+HS combined vaccine shots under routine managemental practice at the farm.

The commercially available combined FMD+HS oil adjuvanted vaccine (containing trivalent FMD inactivated antigens of O, A and Asia-1 strains I.P. and *P. multocida* I.P. adsorbed onto aluminium hydroxide gel; mineral oil as adjuvant, appropriate preservative and diluents), was used in the present study.

The animals in all the three age groups were vaccinated with combined FMD+HS oil adjuvanted vaccine @3 ml through deep intramuscular route. The farm had earlier started vaccinating the animals bi-annually with combined FMD+HS oil adjuvanted vaccine few years back.

Collection of blood, sera separation and transportation: The blood samples (3-5 ml, pre-vaccination and then every month up to 6 months post-vaccination) were collected aseptically, allowed to clot under shade, serum separated in sterile cryovials, transported under cold chain to the laboratory and stored at -20°C till used. The permission for collection of sera samplefrom animals at monthly intervals was granted by Institutional Animal Ethics Committee vide LUVAS/VCC/IAEC/1630-58 dt.26.07.2018.

Single dilution indirect ELISA: The single dilution indirect ELISA (sdiELISA) for detection of serum anti-*P. multocida* Abs, developed in house at the Department of Veterinary Microbiology, COVS, LUVAS, Hisar (Kumar *et al*. 2003) was employed with minor modifications as described. Twenty four sera sample (1:10 dilution, 50 µl) were tested in each ELISA plate in triplicate and 1:10,000 goat anti-mouse IgG horseradish peroxidase (Sigma-Aldrich, Saint Louis, MO, USA) was used as conjugate. Finally, the Ab titres (log_{10}) were calculated by putting the OD value of each serum sample in a regression equation (Kumar *et al*. 2003) developed during the development of sdiELISA as under:

$$\text{Antibody titre } (log_{10})\ Y = a + bX$$

where constant a, 1.35; constant b, 0.05; and X, O.D. value of a test well/ (Mean + 3S.D. value of negative control wells).

The serum samples with Ab titers $log_{10} \geq 1.80$ were interpreted as 'protected'.

Statistical analysis: The statistical analysis of data was performed using SPSS 21.0 version of Microsoft (SPSS, 2001). Two-way analysis of variance (ANOVA) was used to examine the Ab titers of the three experimental groups of immunized buffaloes, and the data were presented as mean standard errors (S.E.). The difference between subclasses of mean was determined using Duncan's multiple range test (DMRT) when the P-values were significant (P<0.05). To evaluate the relationship between an animal's level of protection and its age and the month or months after vaccination, the Pearson Chi square test was used.

RESULTS AND DISCUSSION

The anti-*P.multocida* mean serum Ab titres (log_{10}) ranging from 1.4418-2.2806, 1.4527-2.6283, 1.4394-2.8693, 1.4833-3.0295, 1.4798-3.3018, 1.5103-2.6099 and 1.35-2.6933, respectively were observed for pre-vaccination and subsequently every month up to six month(s) post-vaccination, for all three age groups vaccinated with combined FMD+HS vaccine.

Protection status of animals: The percentage of calves demonstrating protective Ab titres ($\geq log_{10}1.8$) reached peak (60%) at three months post-vaccination but reduced (35%) subsequently at five and six months post-vaccination (Fig. 1). The percentage of heifers demonstrating protective titres reached peak (90%) at five months and reduced (85%) at six months post-vaccination. However, the percentage of adult buffaloes demonstrating protective titres reached peak (95%) at two months post-vaccination and reduced (80%) at six months post-vaccination (Fig. 1). The percentage of protected calves was lower than heifers and adults during pre-vaccination and all the six months post-vaccination (Fig. 1). The percentage of heifers demonstrating protective titres was better than calves. Likewise, the percentage of adult buffaloes (receiving more than five/multiple shots) demonstrating protective titres was highest than calves and heifers except six months post-vaccination.

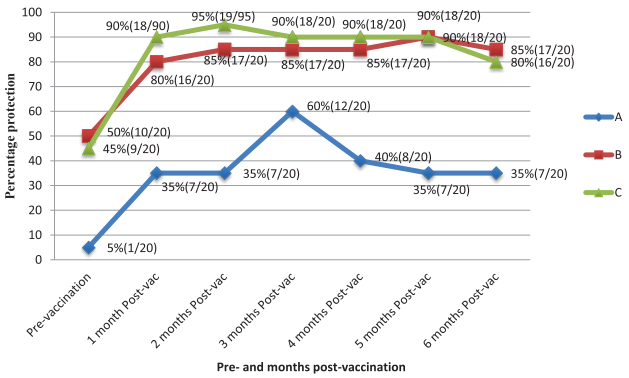

Fig. 1. Percentage of animals demonstrating anti-*P. multocida* protective antibody titres ($\geq log_{10}$ 1.8) in different age groups (n=20) of buffaloes vaccinated with combined FMD+HS vaccine: Group A (calves, <1 year); B (heifers, 1 to 3 years) and C (adults, >3 years).

The heifers and adult buffaloes exihibited protective Ab response up to six months and demonstrated very good herd immunity (>80%) status (Fig.1). However, animals exihibiting protective titres was lower in calves, suggestive of low herd immunity. This may be due to the less potent immune system during initial phase in growing calves and thus calves may need booster vaccinations to exhibit protective antibody titres. Further, these calves had received only 1-2 shots of combined FMD+HS vaccine so far.

Of all the animals demonstrating protective Ab titres in three age groups, maximum (18.28%) animals were at three months post-vaccination (Fig. 2a). Likewise, during

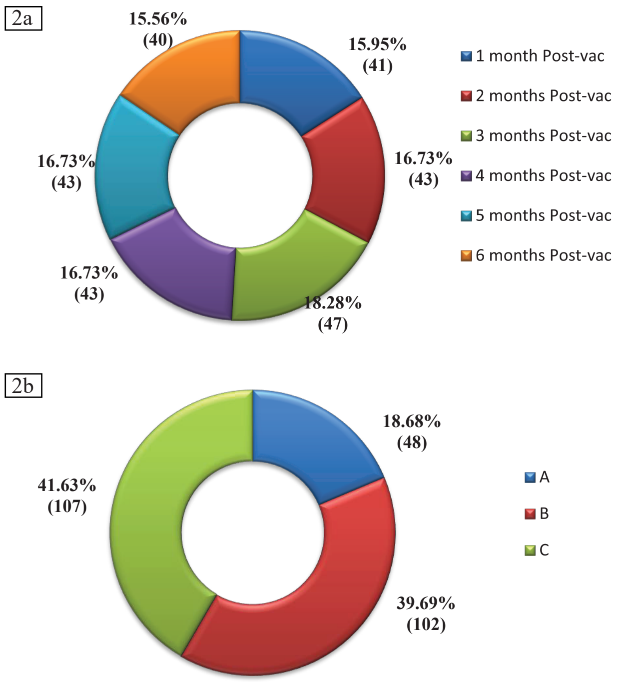

Fig. 2. Percentage of total animals demonstrating anti - *P. multocida* protective antibody titres in animals vaccinated with combined FMD+HS vaccine: (a) one to six month(s) post-vaccination and (b) different age groups : Group A (calves, <1 year); B (heifers, 1 to 3 years) and C (adults, >3 years).

the entire six month(s), maximum (41.63%) were adult buffaloes (Fig. 2b). Of the three age groups, maximum percentage of animals demonstrating protective Ab titres were 25%, 17.65% and 17.76% in calves, heifers and adults (Fig. 3) at three, five and two months post-vaccination, respectively. The peak values were achieved rapidly in adult buffaloes (2 months) than calves (3 months) and heifers (5 months) post-vaccination (Fig 3).

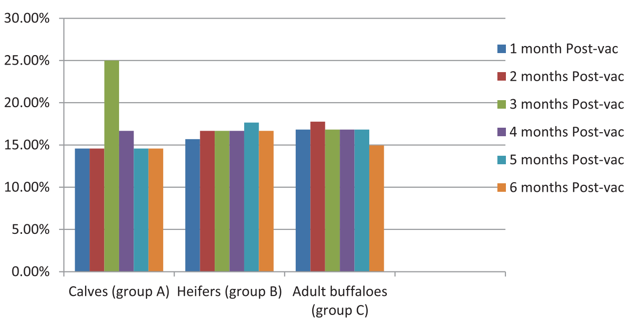

Fig. 3. Month-wise percentage of animals demonstrating anti-*P. multocida* protective antibody titres of the total protected animals in individual group vaccinated with combined FMD+HS vaccine for: Calves-group A, Heifers-group B and Adult buffaloes-group C.

Similar classical pattern of humoral immune response was earlier reported with gradual increase and achievement of peak Ab titers by 42 days and gradual decline by 128 days post-vaccination (Qureshi and Saxena 2014). Bora *et al.* (2019) also reported protective immune response in calves at 28 days post-vaccination with combined FMD virus and *P. multocida* antigens. In another study, protective Ab levels were maintained up to 84 days post-vaccination in buffaloes and declined subsequently (Sangwan 1999). The Ab titers in HS vaccinated buffaloes were protective till four months, but marginally protective at six months post-vaccination (Kumari *et al*. 2007, Markam *et al*. 2009). Likewise, 67.94% HS vaccinated buffaloes demonstrated protective anti-HS antibodies till 90th day post-vaccination (Audarya *et al.* 2020). Maintenance of varied duration of reliable immunity from 3-9 months has earlier been reported in HS vaccinated animals (De Alwis 1999). Combined FMD+HS vaccine produced better antibody titres and maintained for longer duration (Altaf *et al*. 2012). Earlier, combined FMD+HS vaccine has been reported to produce higher antibody titres than FMD alone vaccine in buffalo calves (Chhabra *et al*. 2004). Serological response to FMD+HS combined vaccine at 0, 30, 90 and 180 days post-vaccination in cattle (>4 months age) has been reported to be protective (Prasad *et al*. 2019). Likewise, immune response in cattle vaccinated with FMD+HS+BQ combined vaccine has also been demonstrated to be protective (Reddy *et al*. 1997). Further, use of combined vaccines (FMD+HS or FMD+HS+BQ) was better suited to minimize efforts required in vaccinating animals.

Statistical analysis

Two way ANOVA

Effect of month(s) post-vaccination: The month-wise mean Ab titres demonstrated positive effect of month(s) post-vaccination on Ab titres for particular age group as compared to pre-vaccination titres (Table 1). The F value was significantly higher for all three age groups (P<0.05).

Effect of age of animal: The age group-wise anti-*P. multocida* mean Ab titres demonstrated a positive effect of age for particular month(s) post-vaccination (Table 2). The F value was significantly higher for pre- and all six month(s) post-vaccination (P<0.05) indicating significant difference for all three age groups. There was simultaneous effect of age of animal and particular month(s) post-vaccination on Ab titres.

The calves and heifers demonstrated peak mean Ab titres $\log_{10}1.86\pm0.06$ and 2.33 ± 0.08, respectively at three months post-vaccination. There after Ab titres started declining and maintained up to six months post-vaccination for heifers but not for calves. On the other hand, adult buffaloes demonstrated peak titres $\log_{10}2.06\pm0.05$ two months post-vaccination, and protective titres maintained up to six months post-vaccination. Surprisingly, mean Ab titres were higher in heifers as compared to calves and adults during pre- and post-vaccination. This may be due to more potent immune system during developmental phase in heifers as well as having received 3-5 shots of combined FMD+HS vaccine so far.

Pearson Chi square test

Effect of month(s) post-vaccination: The anti-

Table 1. Month-wise anti- *P. multocida* (Mean±SE) antibody titres (log_{10}) for particular group with respective F and P-value in buffaloes vaccinated with combined FMD+HS vaccine

Pre- and month(s) post- vaccination	Group A		Group B		Group C	
	Mean±SE	F and P-value	Mean±SE	F and P-value	Mean±SE	F and P-value
Pre-vaccination	$1.58^{a}\pm0.03$	3.886^{**}, 0.001	$1.84^{a}\pm0.05$	5.379^{**}, 0.000	$1.78^{a}\pm0.03$	5.386^{**}, 0.000
1 month Post-vac	$1.75^{bc}\pm0.04$		$2.04^{ab}\pm0.06$		$2^{b}\pm0.03$	
2 months Post-vac	$1.7^{ab}\pm0.03$		$2.03^{ab}\pm0.07$		$2.06^{b}\pm0.05$	
3 months Post-vac	$1.86^{c}\pm0.06$		$2.33^{c}\pm0.08$		$2.01^{b}\pm0.04$	
4 months Post-vac	$1.75^{bc}\pm0.04$		$2.23^{ab}\pm0.09$		$2.03^{b}\pm0.05$	
5 months Post-vac	$1.73^{b}\pm0.04$		$2.13^{b}\pm0.06$		$2.02^{b}\pm0.04$	
6 months Post-vac	$1.74^{bc}\pm0.04$		$2.05^{b}\pm0.06$		$1.96^{b}\pm0.04$	

Mean values with different letters in superscript differ significantly at P-value (< 0.05); SE, Standard Error; **, F-value highly significant at P-value (<0.05).

Table 2. Age group-wise anti- *P. multocida* (Mean±SE) antibody titres (log_{10}) for pre- and particular month(s) post-vaccination with respective F and P-value in buffaloes vaccinated with combined FMD+HS vaccine

Age group	Mean±SE antibody titres pre- and different month(s) post-vaccination						
	Pre - vaccination	1 month Post-vac	2 months Post-vac	3 months Post-vac	4 months Post-vac	5 months Post-vac	6 months Post-vac
Group A	$1.58^{a}\pm0.03$	$1.75^{a}\pm0.04$	$1.7^{a}\pm0.03$	$1.86^{a}\pm0.06$	$1.75^{a}\pm0.04$	$1.73^{a}\pm0.04$	$1.74^{a}\pm0.04$
Group B	$1.84^{b}\pm0.05$	$2.04^{b}\pm0.06$	$2.03^{b}\pm0.07$	$2.33^{b}\pm0.08$	$2.23^{c}\pm0.09$	$2.13^{b}\pm0.06$	$2.05^{b}\pm0.06$
Group C	$1.78^{b}\pm0.03$	$2^{b}\pm0.03$	$2.06^{b}\pm0.05$	$2.01^{a}\pm0.04$	$2.03^{b}\pm0.05$	$2.02^{b}\pm0.04$	$1.96^{b}\pm0.04$
F and P value	13.817^{**}, 0.000	12.634^{**}, 0.000	15.010^{**}, 0.000	13.861^{**}, 0.000	15.487^{**}, 0.000	18.106^{**}, 0.000	10.519^{**}, 0.000

Mean values with different letters in superscript differ significantly at P-value (< 0.05); SE, Standard Error;**, F-value is significantly higher at P-value (<0.05).

P. multocida monthly mean Ab titres showed an improvement for a particular group post-vaccination compared to pre-vaccination (Table 3). The Pearson Chi square value for calves, heifers and adults was non-significant (P>0.05), significantly lower (P>0.05) and significantly higher (P <0.05), respectively. This indicated that there was no effect, moderate effect and higher effect of particular month(s) post-vaccination on number of protected calves, heifers and adult buffaloes, respectively.

Table 3. Age group-wise Pearson Chi square and P-value for anti- *P. multocida* antibody titres between pre- and different month(s) post-vaccination in buffaloes vaccinated with combined FMD+HS vaccine

Values	Group A	Group B	Group C
Pearson Chi squarevalue	19.215	13.750^{*}	25.244^{**}
Asymp.Sig. (2-sided) (P-value)	0.083	0.033	0.000

*, non-significant at P-value (>0.01) and significant at P-value (<0.05); **, significantly higher at P-value (<0.05).

Effect of age of animal: The age group-wise mean Ab titres demonstrated positive effect of age for particular month(s) post-vaccination (Table 4). Pearson Chi square value for pre-vaccination and all six months (except 3 months post-vaccination) was significantly higher (P <0.05), indicating that vaccine was not equally effective in all age group of animals for particular month(s) post-vaccination, its effectiveness varied with age of animals. Maximum animals were protected three months post-vaccination for all three age groups.

The present study indicated that combined FMD+HS oil adjuvanted vaccine provided longer duration of immunity against HS in multiple vaccinated adult buffaloes, which also strengthened the previously reported observations by De Alwis (1992). Further, the combined FMD+HS vaccine was found to be effective in buffaloes of all age groups at government organised farm. Thus, the FMD+HS combined vaccination could also be an ideal approach in field conditions under mass vaccination programs leading to reduction in labour inputs, cold chain maintenance, repeated vaccination to animals, saving government exchequer and can be effectively used in controlling the two most dreaded, economic important and endemic diseases, viz. FMD and HS in developing countries including India.

It can further be concluded that the calves may require

Table 4. Month-wise Pearson Chi square and P-value for anti- *P. multocida* antibody production between different groups of buffaloes vaccinated with combined FMD+HS vaccine

Value	Pre- and different month(s) post-vaccination						
	Pre- vaccination	1 month Post-vac	2 months Post-vac	3 months Post-vac	4 months Post-vac	5 months Post-vac	6 months Post-vac
Pearson Chi square value	16.467^{**}	16.358^{**}	20.642^{**}	6.819	15.358^{**}	19.863^{**}	14.938^{**}
Asymp. Sig. (2- sided) (P value)	0.002	0.003	0.000	0.146	0.004	0.000	0.005

**, significantly higher at P-value (<0.01).

booster dose to increase their immunity since higher number of heifers and buffaloes were protected up to six month(s) post-combined FMD+HS vaccination than calves.

To the best of available literature and knowledge, this is the first report on kinetics of Ab titres in buffaloes of different age groups against combined FMD+HS vaccine. The present study will help to understand the formulation of nationwide better vaccination strategies for prevention and control of HS on the pattern of FMD-Control Program.

ACKNOWLEDGEMENTS

The first author is grateful to the Department of Animal Husbandry, Government of Rajasthan for permission and necessary leave to undertake Ph.D. studies at Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar as an in-service candidate. The authors are thankful to the authorities of LUVAS, Hisar and State Cattle Breeding Project-II, Hisar (managed by Haryana Livestock Development Board, Panchkula) for providing the necessary permission and infrastructure facilities for undertaking the study. The help provided by farm officials and laboratory staff in collection of blood samples and processing of serum samples is duly acknowledged.

REFERENCES

Altaf I, Siddique M, Muhammad K, Irshad M, Khan M Z, Anjum A A and Kamran M. 2012. Antibody response of rabbits to combined hemorrhagic septicemia and foot-and-mouth disease virus vaccine. *Journal of Animal Plant Science* **22**: 501–04.

Amanat F, Stadlbauer D, Strohmeier S, Nguyen T H O, Chromikova V, McMahon M, *et al.* 2020. *Nature Medicine* **26**: 1033–36.

Audarya S, Kakker N, Sharda R and Chhabra D. 2020. Immune response to an alum precipitated haemorrhagic septicaemia vaccine in buffaloes at a semiorganized farm of Madhya Pradesh in India. *Turkey Journal of Veterinary Animal Science* **44**: 443–48.

Bora M, Akhila G, Hanumant D, Reddi K V N R, Shebannavar S, Rao T V S and Reddy G S 2019. Immunogenicity of a combined vaccine containing foot and mouth disease virus antigen and *Pasteurella multocida* antigen in bovines. *Journal of Entomology and Zoology Studies* **7**(6): 51–54.

Chhabra R, Sharma R and Kakker N K. 2004. Comparative immunogenicity of foot and mouth disease virus antigens in FMD-HS combined vaccine and FMD vaccine alone in buffalo calves. *Indian Journal of Experimental Biology* **42**: 259–64.

De Alwis M C L. 1992. Haemorrhagic septicaemia–A general review. *British Veterinary Journal* **148**: 99–112.

De Alwis M C L. 1999. *Haemorrhogic septicemia. Australian Center for International Agricultural Research* Monograph no. 57. ACIAR, Canberra, Australia.

Habib F, Jabbar A, Shahnawaz R, Menon A,Yousaf A, Bilal M, Jamil T, Khalil R and Sharif A. 2019. Prevalence of hemorrhagic septicemia in cattle and buffaloes in Tandojam, Sindh, Pakistan. *Online Journal of Animal and Feed Research.* **9**(5): 187–90.

Kumar A, Dahiya G and Kakker N K. 2003. Single dilution ELISA for estimation of serum antibody levels against haemorrhagic septicaemia. *Haryana Veterinarian*. **42**: 51–55.

Kumari L, Kumar S, Kumar A, Mahajan N K, Pander B L and Khokhar R S. 2007. Seromonitoring of cattle and buffaloes vaccinated against haemorrhagic septicaemia using monoclonal antibody based indirect ELISA. *Journal of Immunology and Immunopathology*. **9**: 67–70.

Mahboob S, Iqbal M and Rahman M. 2023. Development of ELISA-based diagnostic methods for the detection of haemorrhagic septicaemia in animals. *Journal of Microbiological Methods* **204**: 106652.

Markam S K, Khokhar R S, Mahajan N K, Kumar A, Kadian S K and Kapoor S. 2009. Monoclonal antibody based ELISA for seromonitoring of bovines vaccinated against haemorrhagic septicaemia in Haryana. *Haryana Veterinarian* **48**: 88–90.

Muenthaisong A, Rittipornlertrak A, Nambooppha B, Tankaew P, Varinrak T, Pumpuang M, Muangthai K, Atthikanyaphak K, Singhla T, Pringproa K, Punyapornwithaya V, Sawada T and Sthitmatee N. 2021. Immune response in dairy cattle against combined foot and mouth disease and haemorrhagic septicemia vaccine under field conditions. *BMC Veterinary Research* **17**(1): 1–12.

OIE. 2021. OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Chapter 3.4.10: Haemorrhagic Septicaemia.

Pal R, Kumar S, Bardhan D, Anandasekaran G, Verma M, Singh G, Chilambarasan M and Singh R K. 2017. Estimation of economic losses due to haemorrhagic septicaemia in Haryana. *International Archives of Applied Sciences and Technology* **8**: 09–13.

Prasad V S, Patel S D, Patel K R, Srinivas K and Thesia M J. 2019. Serological response to foot and mouth disease and haemorrhagic septicaemia combined vaccine in cattle. *IOSR Journal of Agriculture and Veterinary Science* (IOSR-JAVS). **12**: 57–61.

Qureshi S and Saxena H M. 2014. Estimation of titres of antibody against *Pasteurella multocida* in cattle vaccinated with haemorrhagic septicemia alum precipitated vaccine. *Veterinary World* **7**: 224–28.

Reddy G S, Antony P S and Srinivasan V A. 1997. Serological response to combined vaccination of cattle against foot-and-mouth disease, haemorrhagic septicaemia and blackquarter. *Indian Journal of Animal Sciences* **67**: 585–86.

Sangwan V P. 1999. 'Studies on outer membrane protein(s) of *Pasteurella multocida* for monitoring protective immunity.' Master's thesis, Hisar, CCS: Haryana Agricultural University.

Shome R, Deka R P, Sahay S, Grace D and Lindahl J F. 2019. Seroprevalence of hemorrhagic septicemia in dairy cows in Assam, India. *Infection Ecology and Epidemiology* **9**: 1604064.

Tankaew P, Srisawat W, Singhla T, Tragoolpua K, Kataoka Y, Sawada T and Sthitmatee N. 2018. Comparison of two indirect ELISA coating antigens for the detection of dairy cow antibodies against *Pasteurella multocida*. *Journal of Microbiological Methods* **145**: 20–27.