

Persistence of maternal antibodies in calves born of combined foot-and-mouth disease + haemorrhagic septicaemia vaccinated buffaloes at organized dairy farm

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ABSTRACT

Twelve apparently healthy and recently parturient Murrah buffaloes, vaccinated with Foot-and-mouth disease (FMD) + Haemorrhagic septicaemia (HS) combined vaccine at an organized farm along with their newborn calves were induced for detection of antibodies in serum and colostrum against FMD virus (FMDV) serotypes O, A and Asia-1, and *Pasteurella multocida* using enzyme linked immunosorbent assay. The calves born to vaccinated dams showed the presence of protective maternal antibody titre ($\geq 1.8 \log_{10}$) from birth till the period of study (16th week of age) against FMDV serotypes O, A and Asia-1. The maternal antibody titre against *P. multocida* were protective ($\geq 1.8 \log_{10}$) till fifth day of age which became partially protective thereafter till the period of study. It is recommended to avoid vaccination of the calves before 16th week of age with FMD+HS combined vaccine.

Keywords: Buffalo calf, Buffalo colostrum, Buffalo serum, Foot-and-mouth disease virus, Maternal antibodies, *Pasteurella multocida*

Foot-and-mouth disease (FMD) and haemorrhagic septicaemia (HS) are the most common and economically important diseases of cattle and buffaloes. FMD is caused by FMD virus (FMDV) and characterized by high fever, excessive frothy salivation, vesicles formation on the tongue, teats and inter-digital space, decrease in milk production in large ruminants and vesicular presence on the snout with lameness in affected pigs (Audarya 2020). Only three serotypes namely, O, A, and Asia-1 of the seven serotypes present globally have been reported in India. HS is an acute, fatal, bacterial, septicaemic disease caused by *Pasteurella multocida* and characterized by high fever, lethargy, nasal discharge, respiratory distress, oedematous swelling in the throat and brisket region resulting in high mortality and morbidity (OIE 2021).

Mass vaccination is often practiced for the control of FMD and HS (Muenthaisong *et al.* 2021). Government of India had launched FMD-Control Programme (FMD-CP) in 2003-04 and subsequently by National Animal Disease Control Programme (NADCP) in 2019. Haryana has been allowed to use combined FMD+HS vaccine under NADCP w.e.f. April 2019, thus reducing the cost and number of vaccination shots to animals.

The new-borns of ruminants are born immunologically naïve and the immunity is transferred passively to these

calves by ingestion and absorption of antibodies from the dam's colostrum, thus protecting the new-borns from most of the diseases until their immune system develops or they are vaccinated. Further, the maternal antibodies could also block the development of immune response in calves to vaccines (Niewesk 2014). For an effective vaccination program, the information on duration of maternal immunity in calves is very essential and required to be assessed precisely (Akhter *et al.* 2015). The present study describes the persistence of maternal antibodies to FMDV and *P. multocida* in buffalo calves fed with colostrum from their dams vaccinated against combined FMD+HS vaccine.

MATERIALS AND METHODS

Animals, vaccine and IAEC permission: Randomly selected apparently healthy and recently parturient 12 Murrah buffaloes maintained at Buffalo Farm, Department LPM, LUVAS, Hisar and their calves were included in the study. The buffaloes were last vaccinated during late gestation with commercially available combined FMD+HS vaccine ($\geq 3 \text{ PD}_{50}$ for each FMDV serotype O, A and Asia-1) as per the manufacturer's instructions. The buffalo calves were allowed to consume colostrum/ milk *ad lib.* from their respective dams, daily upto 5 days. The permission from IAEC was granted vide No. VCC/IAEC/1714 – 44, dated 28.12.2020.

Sample collection and processing: Blood and colostrum/ milk samples were collected from buffaloes and their calves (only blood) on the day of parturition (day 0) and

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daily up to 5 days post parturition. Pre-colostral blood samples were also collected, if available. In addition, blood was also collected at 2, 4, 6, 8, 10, 12, 14 and 16 weeks of age from these calves.

Serum was separated from blood as per standard protocol, milk samples centrifuged @ 3000×g for 30 min to separate fat and milk serum, stored at -20°C until used and thawed at 37°C before use.

Detection of antibodies against FMDV serotypes O, A and Asia-1: The solid phase competitive ELISA (SPCE) as developed and standardized by ICAR-Directorate of FMD, Bengaluru was used to detect the antibody titre against structural proteins of FMDV serotypes O, A and Asia-1 in serum and milk samples (Biswal *et al.* 2019). The serum samples showing log₁₀ antibody titre ≥1.8 were interpreted as having “protective antibody titre” and <1.79 as “non-protective”.

Detection of antibodies against *Pasteurella multocida*: The single dilution indirect ELISA (sdiELISA) developed in Department of Veterinary Microbiology (Kumar *et al.* 2003) was used to detect the antibody titre in serum and milk samples against *P. multocida* with minor modifications (Kumar *et al.* 2021). The final results were calculated as log₁₀ antibody titre and interpreted as protective, partially protective and non-protective if samples were having log₁₀ antibody titre ≥1.80, 1.50-1.79 and ≤1.49, respectively.

Statistical analysis: The data were statistically analysed using SPSS 20.0 software. One way ANOVA was used to determine if there was any significant (p<0.05) difference in antibody titre between buffalo serum, buffalo colostrum/milk and buffalo calf serum against FMDV serotypes O, A and Asia-1, and *P. multocida*. One way ANOVA with repeated measures was used to determine the significant difference (p<0.05) in antibody titres between days for buffalo serum, buffalo colostrum/ milk and buffalo calf

serum. The difference in serum antibody titres between pre-colostral and post-colostral serum samples of buffalo calves was determined using paired ‘t’ test. The associations between antibody titre in buffalo serum, buffalo colostrum/milk and buffalo calf serum against FMDV serotypes O, A and Asia-1 and *P. multocida* were determined using correlation analysis.

RESULTS AND DISCUSSION

The mean antibody titre (log₁₀) against FMDV serotypes O, A and Asia-1 in buffalo calf serum at different time points from day 0 to 5 and week 2 to 16 showed significant (p<0.05) difference (Table 1) and were significantly (p<0.05) higher in calves on second week, from day 1 to second week and from day 2 to second week after birth, respectively.

For all the three FMDV serotypes, the mean antibody titres (log₁₀) in buffalo calves started declining from fourth week onwards and became significantly (p<0.05) lower at 16th week (till the period of study), however, the titres remained protective (≥1.8 log₁₀) till 16th week. These findings were in agreement with Chahe *et al.* (2009) who reported the highest FMDV neutralizing titre at 15 days of age in calves using virus neutralization test. Akhter *et al.* (2015) demonstrated protective level of maternal antibodies against FMDV up to 23 weeks of age in calves born to vaccinated cows. Similarly, Mini *et al.* (2016) demonstrated protective maternal antibodies in calves up to 150 days against FMDV serotype O and Asia-1 and up to 90 days against serotype A. Sareyyüpoğlu *et al.* (2019) reported high maternal antibody titre till 120 days in calves born to cows vaccinated against FMDV at late gestation as compared to calves born of cows vaccinated at early gestation.

The mean antibody titre (log₁₀) against *P. multocida* in

Table 1. Log₁₀ maternal antibody titres (Mean±S.E.) against FMDV serotypes O, A and Asia-1 and *P. multocida* in serum of buffalo calves born to dams vaccinated with FMD+HS combined vaccine

Age of calf	FMDV O	FMDV A	FMDV Asia-1	<i>P. multocida</i>
Day 0 (pre-colostral)	1.40 ^a	1.40 ^a	1.40 ^a	1.30±0.01 ^a
Day 0 (post-colostral)	2.33±0.14 ^{ABb}	2.33±0.14 ^{ABb}	2.33±0.14 ^{ABb}	1.85±0.10 ^b
Day 1	2.39±0.05 ^{AB}	2.35±0.05 ^B	2.40±0.04 ^{AB}	1.87±0.07
Day 2	2.37±0.06 ^{AB}	2.39±0.05 ^B	2.44±0.03 ^B	1.87±0.07
Day 3	2.37±0.06 ^{AB}	2.42±0.04 ^B	2.44±0.03 ^B	1.84±0.06
Day 4	2.39±0.06 ^{AB}	2.44±0.03 ^B	2.46±0.03 ^B	1.83±0.07
Day 5	2.39±0.06 ^{AB}	2.46±0.03 ^B	2.44±0.03 ^B	1.82±0.07
Week 2	2.41±0.04 ^B	2.38±0.06 ^B	2.43±0.04 ^B	1.70±0.05
Week 4	2.31±0.06 ^{AB}	2.31±0.06 ^{AB}	2.33±0.07 ^{AB}	1.65±0.05
Week 6	2.26±0.07 ^{AB}	2.23±0.08 ^{AB}	2.21±0.08 ^{AB}	1.65±0.05
Week 8	2.30±0.08 ^{AB}	2.23±0.08 ^{AB}	2.25±0.09 ^{AB}	1.63±0.05
Week 10	2.30±0.08 ^{AB}	2.18±0.08 ^{AB}	2.20±0.09 ^{AB}	1.61±0.05
Week 12	2.02±0.11 ^{AB}	2.23±0.10 ^{AB}	2.15±0.10 ^{AB}	1.61±0.05
Week 14	2.15±0.12 ^{AB}	2.16±0.12 ^{AB}	2.20±0.12 ^{AB}	1.61±0.03
Week 16	2.02±0.11 ^A	1.94±0.16 ^A	2.05±0.15 ^A	1.62±0.03

Mean±S.E. bearing different superscript (A, B) differ significantly (p<0.05) between day/week; Mean±S.E. bearing different superscript (a, b) differ significantly (p<0.05) between pre-colostral and post-colostral serum samples.

buffalo calf serum at different points from day 0 to 5 and week 2 to 16 showed no significant ($p>0.05$) difference (Table 1). The protective titre ($\geq 1.8 \log_{10}$) in calves against *P. multocida* was maintained till day 5 post parturition which became partially protective thereafter till the period of study (16th week). The mean antibody titre started declining between week 2 to 4 and remained almost similar during week 8 to 16. These findings were in agreement with El-Eragi *et al.* (2001) who observed decrease in maternal antibody titre between 2 and 4 weeks of age and no significant change in antibody titre from week 8 to 16 against *P. multocida* in Holstein-Friesian calves born to dams vaccinated against HS. They also observed a decrease in antibody titre after week 16 and therefore recommended vaccine during 4 to 6 months of age in calves.

The statistical analysis of comparison of mean antibody titres ($\log_{10}$) against FMDV serotype O, A and Asia-1, and *P. multocida* revealed that the titres ($\log_{10}$) were significantly ($p<0.05$) different and higher in serum of calves after colostrum feeding than in pre-colostrum consumption (Table 1). The mean antibody titres in buffalo calf serum, pre- and post-colostrum feeding were 1.40 and 2.33, respectively against all the three FMDV serotypes. Similarly, the antibody titres against *P. multocida* were 1.30 and 1.85, pre- and post-colostrum feeding, respectively. These findings are in agreement with the observations of El-Eragi *et al.* (2001), Chahe *et al.* (2009) and Mini *et al.* (2016).

There was no significant difference ($p>0.05$) in mean antibody titre between buffalo serum, colostrum/ milk and calf serum at day 1 and 2 when compared with other days against all the three FMDV serotypes (Tables 2-4), although the buffalo colostrum/ milk demonstrated slightly higher titre than buffalo and calf serum. The mean $\log_{10}$ antibody titre in buffalo colostrum/ milk was significantly ($p<0.05$) lower from day 3 onwards in comparison to dam's and calf's serum. The calf serum showed slightly higher titres than its dam's serum. For *P. multocida*, there

was a significant ($p<0.05$) difference in mean antibody titre ($\log_{10}$) between buffalo serum, colostrum/ milk and calf serum on day 0, 2, 3, 4 and 5 (Table 5). The titre in colostrum/ milk was significantly higher on day 0 and lower from second to fifth day, than in dam and calf serum. However, no significant difference was observed in antibody titres on day 1 between the buffalo serum, colostrum/ milk and calf serum. The buffalo serum showed slightly higher titres than buffalo calf serum on all the days against *P. multocida*. These findings were in agreement with Chahe *et al.* (2009) and Mauncell (2014). Chahe *et al.* (2009) found higher virus neutralizing antibody (VNA) titres in calves than corresponding dams against FMDV, probably due to active pumping of antibodies in colostrum after parturition. Mauncell (2014) observed lower blood VNA titres in dams due to production of colostrum 4 to 6 weeks prior to parturition in udders and transfer of maternally derived antibodies from blood to the colostrum. Additionally, the statistical analysis revealed no significant ($p>0.05$) difference in mean antibody titre ($\log_{10}$) in dam and calf serum between the days (0 to 5) against all the three FMDV serotypes and *P. multocida*, with buffalo calf serum demonstrating slightly lower antibody titre on day 0, which increased thereafter. The mean antibody titres ($\log_{10}$) in buffalo colostrum/ milk were significantly ($p<0.05$) higher on day 0 to 2, started decreasing from second day onwards and were significantly lower on 5th day against FMDV serotypes and *P. multocida*. The mean antibody titre ($\log_{10}$) was protective (≥ 1.8) in buffalo serum and calf serum at day 0, upto fifth day against FMDV serotypes O, A and Asia-1 and *P. multocida*. However, the mean antibody titre ($\log_{10}$) in buffalo colostrum/ milk was protective on the day 0, till second day post parturition and non-protective from third to fifth day against FMDV serotypes. Additionally, the mean antibody titre ($\log_{10}$) in buffalo colostrum/ milk was protective on the day 0 and 1, partially protective on day 2 and non-protective from day 3 to 5 day against *P. multocida*.

Table 2. $\log_{10}$ antibody titres (Mean $\pm$ S.E.) against FMDV serotype O in serum and milk samples of buffaloes (n=12) and in serum samples of their calves (n=12)

Group	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Buffalo serum	2.25 $\pm$ 0.07	2.23 $\pm$ 0.07	2.21 $\pm$ 0.06 ^{ab}	2.22 $\pm$ 0.07 ^b	2.26 $\pm$ 0.06 ^b	2.28 $\pm$ 0.07 ^b
Buffalo colostrum/ milk	2.43 $\pm$ 0.05 ^D	2.32 $\pm$ 0.07 ^D	2.06 $\pm$ 0.10 ^{aC}	1.66 $\pm$ 0.08 ^{aB}	1.55 $\pm$ 0.05 ^{aAB}	1.51 $\pm$ 0.05 ^{aA}
Buffalo calf serum	2.33 $\pm$ 0.09	2.39 $\pm$ 0.05	2.37 $\pm$ 0.06 ^b	2.37 $\pm$ 0.06 ^b	2.39 $\pm$ 0.06 ^b	2.39 $\pm$ 0.06 ^b

Mean $\pm$ SE bearing different superscript (a, b) differ significantly ($p<0.05$) in the same column; Mean $\pm$ SE bearing different superscript (A, B, C, D) differ significantly ($p<0.05$) in the same row.

Table 3. $\log_{10}$ antibody titres (Mean $\pm$ S.E.) against FMDV serotype A in serum and milk samples of buffaloes (n=12) and in serum samples of their calves (n=12)

Group	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Buffalo serum	2.24 $\pm$ 0.08	2.24 $\pm$ 0.08	2.23 $\pm$ 0.07 ^b	2.24 $\pm$ 0.08 ^b	2.25 $\pm$ 0.07 ^b	2.25 $\pm$ 0.07 ^b
Buffalo colostrum/ milk	2.39 $\pm$ 0.06 ^D	2.25 $\pm$ 0.08 ^D	2.00 $\pm$ 0.10 ^{aC}	1.62 $\pm$ 0.07 ^{aB}	1.57 $\pm$ 0.05 ^{aAB}	1.53 $\pm$ 0.05 ^{aA}
Buffalo calf serum	2.33 $\pm$ 0.09	2.35 $\pm$ 0.05	2.39 $\pm$ 0.05 ^b	2.42 $\pm$ 0.04 ^b	2.44 $\pm$ 0.03 ^c	2.46 $\pm$ 0.03 ^c

Mean $\pm$ SE bearing different superscript (a, b, c) differ significantly ($p<0.05$) in the same column; Mean $\pm$ SE bearing different superscript (A, B, C, D) differ significantly ($p<0.05$) in the same row.

Table 4. Log₁₀ antibody titres (Mean±S.E.) against FMDV serotype Asia-1 in serum and milk samples of buffaloes (n=12) and in serum samples of their calves (n=12)

Group	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Buffalo serum	2.28±0.07	2.26±0.06	2.30±0.06 ^b	2.28±0.07 ^b	2.28±0.06 ^b	2.28±0.06 ^b
Buffalo colostrum/ milk	2.46±0.03 ^E	2.28±0.07 ^D	1.95±0.11 ^{aC}	1.60±0.08 ^{aB}	1.58±0.06 ^{aAB}	1.51±0.07 ^{aA}
Buffalo calf serum	2.33±0.09	2.40±0.04	2.44±0.03 ^b	2.44±0.03 ^b	2.46±0.03 ^c	2.44±0.03 ^b

Mean±SE bearing different superscript (a, b, c) differ significantly ($p < 0.05$) in the same column; Mean ± SE bearing different superscript (A, B, C, D) differ significantly ($p < 0.05$) in the same row.

Table 5. Log₁₀ antibody titres (Mean±S.E.) against *P. multocida* in serum and milk samples of buffaloes (n=12) and in serum samples of their calves (n=12)

Group	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Buffalo serum	1.90±0.02 ^a	1.91±0.02	1.92±0.02 ^b	1.92±0.02 ^b	1.92±0.02 ^b	1.91±0.02 ^b
Buffalo colostrum/ milk	2.24±0.10 ^{bE}	1.97±0.09 ^D	1.69±0.07 ^{aC}	1.46±0.04 ^{aB}	1.40±0.03 ^{aA}	1.38±0.03 ^{aA}
Buffalo calf serum	1.85±0.08 ^a	1.87±0.07	1.87±0.07 ^b	1.84±0.06 ^b	1.83±0.07 ^b	1.82±0.07 ^b

Mean±SE bearing different superscript (a, b) differ significantly ($p < 0.05$) in the same column; Mean ± SE bearing different superscript (A, B, C, D & E) differ significantly ($p < 0.05$) the same row.

A positive correlation was observed between mean antibody titre (log₁₀) in buffalo serum and buffalo colostrum/ milk; buffalo colostrum/ milk and buffalo calf serum; buffalo serum and buffalo calf serum at different time points against FMDV serotypes O, A and Asia-1, and *P. multocida* on the day of parturition and up to five days; and were in agreement with Bhanot *et al.* (2015) and Sareyyüpoğlu *et al.* (2019). Bhanot *et al.* (2015) demonstrated a significant correlation between serum and milk antibody titre of buffaloes vaccinated with FMDV vaccine. Sareyyüpoğlu *et al.* (2019) observed a positive correlation between the colostrum IgG content of dams vaccinated with FMDV vaccine in late gestation and IgG of their calves.

Based on the findings in the present study, it can be inferred that the mean antibody titres in buffalo calves against FMDV serotypes and *P. multocida* were non-protective before colostrum feeding and a sharp increase in antibody titres (protective) were observed in buffalo calf serum post-colostrum consumption, suggesting the transfer of maternal antibodies through colostrum. The antibody titres in colostrum/ milk showed significant difference and were higher for the first two days, declined thereafter as non-protective, suggesting the importance of colostrum consumption by calves at birth to protect them from diseases like FMD and HS until the development of their own immune system. Hence, the colostrum should be fed as early as possible to the calves since quantity of the maternal antibodies against FMDV serotypes O, A and Asia-1 continuously decreases with time, being highest on the day of parturition. The protective maternal antibody titres against FMDV serotypes O, A and Asia-1 were maintained in buffalo calves born to dams vaccinated with combined FMD+HS vaccine till the period of study, i.e. 16 weeks of age. However, for HS, the maternal antibody titres were protective till 5th day of age in buffalo calves and declined to partially protective level from week 2 to

16 (till the period of study). Therefore, the vaccination of buffalo calves with combined FMD+HS vaccine should be avoided before 16th weeks of age. However, further studies are required to determine the exact time of exhaustion of these maternal antibodies in buffalo calf serum.

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