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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(11): 1407-1410

www.thepharmajournal.com
Received: 13-09-2022
Accepted: 17-10-2022

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Effect of FMD vaccination on differential leukocyte count of Murrah, Tharparkar and Vrindavani calves

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Abstract
In the present study, the effects of trivalent oil adjuvant Foot and Mouth Disease (FMD) vaccination was evaluated on the haematological response (different leukocyte %) of naïve calves. A total of 18 healthy female naïve calves, 6 from each breed over 4 months of age were selected for the experimental purpose. Blood samples were collected on-1 (Prevaccination), 1, 7, 14, 28 days post vaccination (DPV). Results revealed that leukocyte % and neutrophil % of Murrah calves were significantly ($P \leq 0.05$) affected by the administration of FMD vaccination whereas it was not significant in Tharparkar and Vrindavani calves. However, vaccination has no effect on lymphocyte %, monocyte %, eosinophil % and basophil % of calves of all the three breeds. From the above study it can be concluded that there is neutrophilia in the calves which may be due to stress related to vaccination.

Keywords: FMD, DLC, Murrah, Tharparkar, Vrindavani

Introduction
India has highest livestock in the world with population of 535.78 million in which cattle and buffalo accounts for 302.34 million (DAHD&F, 2019) [2]. The production of livestock adds 4.11% to the country's GDP and 25.6% to the agricultural GDP. In India, 16% of small farm households' income comes from livestock, which provides a living for about 20.5 million people (Vikaspedia, 2022) [3]. Various animal diseases exhibit significant threat to livestock sector throughout the world in terms of economic devastation as well as loss of livelihood of poor farmers. Due of the dairy industry's fragmented infrastructure, prevention of infectious disease in cattle is a challenging task. Foot and mouth disease (FMD) continues to be the most common and devastating disease affecting cattle production in Indian subcontinent (Richeson *et al*., 2019) [10]. FMD virus (FMDV) is a solitary member of genus Aphthovirus under *Picornaviridae* family (Mann and Sellers, 1989) [7] containing a single-stranded positive sense genomic RNA within icosahedral protein particles without an envelope. In India vaccination strategy is the main control procedure to control FMD. National animal disease control programme (NADCP) was introduced by the Hon'ble Prime Minister in September 2019 for control of FMD and Brucellosis by vaccinating 100% of the cattle, buffalo, sheep, goat, and pig population for FMD and 100% of the bovine female calves of 4-8 months for Brucellosis with total funding of Rs. 13,343 cores for a period of five years (2019-20 to 2023-24). We could better understand the effects of FMD vaccination in calves of different breeds if we keep track of changes in haematology around the time of vaccination. However, there is scanty information about the adverse impacts of FMD vaccination on calves. Therefore, the current research is designed for investigating whether vaccination against FMD causes any alteration in different leukocyte levels on different days of sampling with respect to vaccination. This study was formulated with the objective to estimate the alterations in differential leukocyte count in calves of Murrah, Tharparkar and Vrindavani post FMD vaccination.

Materials and Methods
Location of work
Animal experiments and data collection for the present study was carried out at Cattle and buffalo farm of IVRI, Izatnagar. Laboratory studies were carried out at DFMD, Mukteswar campus as well as at IVRI, Izatnagar campus. The institute is situated at an altitude of 564' above mean sea level, at latitude 28.220N and longitude 79.220E. The location's climatic conditions range from frigid (winter temperatures are approximately 5 °C) to hot (summer temperatures are approximately 47 °C).

Between 15 and 85%, the relative humidity is present. The majority of the yearly rainfall, around 90 to 120 cm, falls between July and September. Through 165 hectares of well-irrigated land of the institute, green fodder supplies were guaranteed throughout the year.

Experimental animals

For the present study total 18 apparently healthy naive calves were selected from two breed of cattle i.e., Vrindavani (50-75% exotic inheritance of Holstein-Friesian, Jersey and Brown Swiss and 25-50% from indigenous Haryana breed), Tharparkar and one breed of buffalo (Murrah), each group with 6 animals (n=6). Calves were above 4months of age without any vaccination and disease. While being weaned, calves were given milk replacer, hay, and small amounts of greens. Ad lib clean, fresh water was provided to the experimental animals throughout the day. Calves were kept in loose housing system and sheds were cleaned twice daily.

Experimental design

Commercially available trivalent FMD vaccine (Raksha Ovac-Trivalent) containing tissue culture virus strains O, A, Asia 1 and inactivated with Azaridine compound, mineral oil as adjuvant was used for the experiment. It was administered as deep intramuscular injection @2 ml to calves. Blood samples were collected from all experimental animals on day-1(24hr before vaccination), day 1, day 7, day 14, day 28 after vaccination by use of syringe from jugular vein and collected in heparinised tubes for assessment of haematological parameters.

Estimation of differential leukocyte count (DLC)

By using Giemsa stain method differential leukocyte count was estimated from the blood sample of both adult female animals and naïve calves. A pre cleaned glass slide was taken and a drop of blood was placed over it. By using another slide (spreader) a thin smear was prepared making an angle 45° with the former slide. Then the smear was air dried and fixed with ethanol (absolute 99.9%) and keep for 3-4minutes, followed by Giemsa staining. First the air dried slide was kept in the staining rack then diluted stain (1:20) was poured over the slides covering the smears. After 1hr wash with tap water. Then keep it on slide drying rack for drying followed by examining under oil immersion objective to determine the % of different types of leukocytes i.e. granulocytes (neutrophils, basophils, eosinophils) and agranulocytes (monocytes and lymphocytes) basing on the structure of nucleus.

Statistical method used

Descriptive statistics by IBM SPSS Statistics 22 was used for calculation of mean for different parameters for both naive calves and lactating cows and the results were expressed as mean± SE. For analyzing the effect of breeds and days on different parameters Repeated Measures ANOVA was used. The following General Linear Model was used for the analysis.

$$y_{ijk} = \mu + G_i + D_j + (GD)_{ij} + e_{ijk}$$

Where

y_{ijk} = k-th observed value of the response variable for i-th breed, j-th day

μ = General mean effect

G_i = Effect of i-th breed

D_j = Effect of the j-th day

$(GD)_{ij}$ = Interaction of i-th group and j-th day

e_{ijk} = error term

The multiple comparisons between breeds, days and interaction for different parameters were done by using Tukey test at 5% level of significance. The analysis was done by JMP 9.0 software. Graphs were plotted for different parameters using Graphpad Prism 5.0 software.

Results and discussions

DLC (%) i.e., total white blood cells, lymphocytes, monocytes, neutrophils, eosinophils and basophils of calves were studied during the pre and post vaccination period on day -1, 1, 7, 14 and 28 dpv in response to FMD vaccination. FMD vaccination had significant ($P \leq 0.05$) effect on lymphocyte %, enhancement in lymphocyte % was obtained during the post vaccination days as compared to the pre vaccination day in Murrah calves and peak was on day 28 DPV. When comparison was made breedwise for the changes in lymphocyte % of calves we found that, Tharparkar showed significantly ($P \leq 0.05$) higher lymphocyte % as compared to the calves of Vrindavani and Murrah. Administration of trivalent FMD vaccination had no significant effect on the lymphocyte % of calves with respect to breeds (P=0.077) and days (P=0.217). Accordingly, no interaction was observed between breeds and days for lymphocyte % of calves (P=0.295).

No significant ($P \geq 0.05$) difference was observed for monocytes% within group and between breeds in response to vaccination in naïve calves. Similar findings were obtained for basophil % and eosinophils %. Administration of trivalent FMD vaccination had no significant effect on the monocytes%, basophil % and eosinophils % of calves with respect to breeds and days. Accordingly, no interaction was observed between breeds and days for the said parameters of calves.

Investigations for neutrophils % in response to FMD vaccination showed significantly ($P \leq 0.05$) higher numbers on 1dpv as compared to the pre vaccination day in Murrah buffalo calves. However, the effect of vaccination on neutrophils % in Vrindavani and Tharparkar calves were found to be non significant ($P \geq 0.05$). Breedwise comparison showed significantly ($P \leq 0.05$) higher numbers of neutrophils in Vrindavani calves followed by Murrah and Tharparkar calves. Administration of trivalent FMD vaccination had no significant effect on the neutrophils % of calves with respect to breeds (P=0.054) and days (P=0.288). Accordingly, no interaction was observed between breeds and days for neutrophils % of calves (P=0.214).

Differential leukocyte count (DLC) is consisting of the total number of leukocytes i.e. neutrophil, eosinophil and basophil (granulocytes); monocytes, and lymphocytes (agranulocytes) (Kraft, 2005; Minervino *et al*., 2009) [6, 8]. It is observed that in cattle leukocyte count tends to decrease with age. Lymphocyte being the major subpopulation varies with age. DLC can be used for diagnostic purposes, general health evaluations, disease monitoring, for the observation of therapy effects etc. In presence of stress, excitement, fear, exercise and during parturition there is physiological leukocytosis. Leukocytosis induced in response to stress is generally indicated by neutrophilia, lymphocytopenia,

eosinopenia, and occasionally monocytosis which is prompted by corticosteroid (Kraft, 2005; Jones and Allison, 2007; Tornquist and Rigas, 2010) [6, 5, 13]. Leukopenia is associated with viral infections, circulatory shock, peracute inflammation, cytotoxic substances, as well as hematopoietic stem cell disorders and bone marrow atrophy (Webb and Latimer, 2011) [14]. Neutrophilia is associated with inflammation, neoplasia, intoxication, endocrine disorders, hemorrhage, and hemolysis etc. (Kraft, 2005; Webb and Latimer, 2011) [6, 14]. Basophilia is associated with hyperlipidemia, allergies, ulcerations; however, basopenia is not reported in literature as basophil reference intervals frequently include zero (Webb and Latimer, 2011) [14]. Eosinophilia is observed in type I (immediate) hypersensitivity reactions and parasitic infection and Eosinopenia may be observed in case of infectious diseases, uremia, and acute hemolysis (Kraft, 2005) [6]. Generally, it is observed that in cattle Monocyte numbers are inconsistent, hence it is not considered as a specific indicator for diseases (Jones and Allison, 2007) [5].

In the present study we investigated DLC (%) of calves during the pre and post vaccination period. Our findings are supported by the reports of following studies. Shawky *et al*. (2016) [12] found that vaccination regime had effect on total leukocytic count in vaccinated groups with neutrophilia and lymphocytosis. A study on Korean goats presented by Jo and coworkers in 2014 showed leukocytosis in FMD-vaccinated group when compared to non-FMD vaccinated group as we found in our study. According to a study by Seo and coworkers in 2019 FMD vaccination increased WBCs, neutrophils, lymphocytes in vaccinated group. Our results are consistent with a previous study that reveled leukocytosis and N:L ratio after vaccination in ruminants (Paape., 2003) [9]. Cha and coworkers in 2016 showed changes in hematological parameters in cattle and pigs post FMD vaccination which supports our study. Neutrophils due to their phagocytic actions are considered as the 1st line of defense in immune system of body against the antigens (Paape, 2003) [9]. These changes in leukocytes in our study may be due to the FMD vaccination induced inflammatory reaction which leads to leukocytosis due to lymphocytosis and neutrophilia during the early periods of vaccination. The neutrophilia obtained in this study may be associated with the stress response due to due to vaccination. The breed wise difference and lack of significance within the group may be due to some underlying health issues, genetic variance of farm animals individually and breedwise manner which caused the variation in their performance. However, role of environmental and managerial factors cannot be ignored. However, further work is required to elucidate the possible roles of leukocytes during the post vaccination period with large number of samples in different farms.

Table 1: DLC (%) of calves during pre and post vaccination period

		Day -1	Day 1	Day 7	Day 14	Day 28
Murrah	Lymphocyte	69.33^{ABc}±1.41	67.83^{ABc}±0.98	74.17^{ABb}±1.22	75.67^{ABab}±1.33	79.00^{ABa}±2.24
	Monocyte	1.17±0.48	1.67±0.49	1.83±0.91	1.83±0.70	1.33±0.67
	Basophil	0.00±0.00	0.17±0.17	0.00±0.00	0.00±0.00	0.17±0.17
	Eosinophil	2.17^{a}±1.05	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.50^{b}±0.50
	Neutrophil	27.17^{ABab}±0.79	30.33^{ABa}±0.88	25.17^{ABb}±0.91	23.17^{ABbc}±1.11	19.83^{ABc}±2.68
Tharparkar	Lymphocyte	76.00^{A}±3.00	71.50^{A}±3.19	79.17^{A}±2.80	76.50^{A}±2.97	72.67^{A}±4.44
	Monocyte	2.50±0.85	2.33±1.09	1.33±0.80	2.50±0.56	2.17±0.75
	Basophil	0.17±0.17	0.00±0.00	0.17±0.17	0.17±0.17	0.17±0.17
	Eosinophil	0.33±0.21	0.00±0.00	0.33±0.21	0.00±0.00	0.17±0.17
	Neutrophil	20.83^{B}±2.12	26.33^{B}±3.15	19.00^{B}±2.16	20.50^{B}±2.42	25.83^{B}±4.25
Vrindavani	Lymphocyte	73.83^{B}±2.81	71.17^{B}±4.93	76.67^{B}±2.28	75.17^{B}±0.87	75.67^{B}±2.49
	Monocyte	2.67^{a}±0.67	2.17^{ab}±0.87	1.83^{ab}±0.60	2.00^{ab}±0.52	0.50^{b}±0.50
	Basophil	0.00±0.00	0.00±0.00	0.00±0.00	0.17±0.17	0.00±0.00
	Eosinophil	0.17±0.17	0.00±0.00	0.33±0.21	0.17±0.17	0.17±0.17
	Neutrophil	23.50^{A}±2.78	27.00^{A}±4.52	21.17^{A}±2.46	22.50^{A}±1.06	24.17^{A}±2.47

Means bearing different superscripts (A, B, C, D) in a column and superscripts (a, b, c, d) in a row differs significantly (p<0.05)

Conclusions

It can be concluded that FMD vaccination has significantly affected the lymphocyte% and neutrophil% of calves whereas there is no effect of vaccination on monocyte%, eosinophil%, basophil% of calves. This may be due to presence of stress related to vaccination. Some strategical ameliorative measures such as feeding supplements with antioxidant property (Vitamin C, Vitamin E, Se), application of oral electrolyte therapy, good management and handling practices should be adopted to reduce stress and to enhance the welfare of calves.

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