



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(6): 1233-1235
Received: 25-09-2018
Accepted: 27-10-2018

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Determination of immunoglobulin G (IgG) concentration in buffalo colostrum and serum of new born calves by indirect ELISA

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Abstract

The present investigation was carried to measure IgG concentrations in Murrah buffaloes and their respective new born calves. Twenty three newly parturated Murrah buffaloes and their respective new born calves, maintained at Instructional Dairy Farm (IDF), G. B. Pant University of Agriculture and Technology, Pantnagar were used in this study. The indirect ELISA was performed to measure the IgG concentrations in the colostrum (first milk) of dams and serum of their new born calves (6 to 12 hrs post birth). Our results showed that IgG concentration in colostrum of dams and serum of their new born calves were varies from 11 to 122.1 mg/ml with a mean of 56.98 ± 5.71 mg/ml and 4.2 to 14.88 mg/ml with a mean of 11.23 ± 0.70 mg/ml, respectively. The results of present finding concluded that, estimation of IgG level helps in prediction of health status of dams and calf at an early age.

Keywords: IgG, indirect ELISA, Murrah buffaloes, new born calves

Introduction

Colostrum is the most precious gift the neonates receive from their mother. Maternal immunoglobulins present in colostrum provide passive immunity to newborn from invading pathogens (Larson *et al.*, 1980) [12]. Guts of neonatal calves are more absorptive during initial phase of life. IgG concentration in colostrum and permeability of colostrum through gut of neonates promptly reduces after 48 hours of birth of calves (Bush and Staley, 1980; Moore *et al.*, 2005) [4, 15].

The colostrum immunoglobulins are similar in composition as present in the mucosal secretions or blood (Duncan *et al.*, 1972) [9]. The bovine IgG are basically categorized into two types e.g. IgG1 and IgG2. Type one immunoglobulin (IgG1) accounts for 80 per cent of the total absorption of Immunoglobulin's by newborn calves (Blom, 1982; Duncan *et al.*, 1972) [3, 9]. Group of proteins, having the protective property against infections are classified into several groups viz.-IgM, IgA, IgG, IgE and IgD (Mix *et al.*, 2006) [14]. IgG is found to be present in ruminant milk, while IgA is present in human milk (Stewlagen *et al.*, 2009) [16]. Different authors found variation in immunoglobulin concentration, as 67.2 mg/ml IgG concentration in bovines (Zhang *et al.* 2001) [18]. The average value of IgG level was found to be 54.0 mg/ml estimated by ELISA test, (Dang *et al.*, 2009) [8] in Murrah buffaloes. Based on morbidity and mortality data, passive transfer is generally considered adequate if the concentration of IgG in serum is ≥ 10.0 g/L at 1-7 days of age (Virtala *et al.*, 1999) [17], although figures as high as 20.0 g/L have been suggested (Chigerwe *et al.*, 2015) [7]. Many authors indicate that the mortality in cattle and buffalo calves ranged from 29.1 % to 39.8% (Afzal *et al.*, 1983) [1], whereas Martin and Wiggin (1973) [13] estimated that 20 % calf mortality resulted in a reduction of 38 % profit of a livestock farm.

The IgG concentration shows a declining trend in calf serum depending on the time of colostrum feeding. So the time of colostrum feeding to a newborn calf is very crucial in initial phase of life just after parturition. Assessment of IgG level in calf serum could be a predictive management tool for the health status (mortality and morbidity) of newly born calf. Depending on the level of IgG in calf serum, animal breeder and farm manager may plan and provide extra care to newborn calves to increase the survivability of calf. So the present study was designed to access the IgG level in buffalo calf serum as well as in dam's colostrum.

Material and Method

Twenty three Murrah buffaloes (after parturition during the period from September 2015 to March 2016) and their respective newly born calves maintained at Instructional Dairy Farm

(IDF), G.B. Pant University of Agriculture and Technology, Pantnagar were taken for the study.

Collection of Serum: From new born buffalo calves, 6 to 12 hrs after the first feeding of colostrum, 3 ml blood from each calf was collected in vacutainer tubes without anticoagulant and was kept in a slant position for isolation of serum.

Collection of Colostrum Samples: Before feeding to calf 10 ml of fresh colostrum sample from each dam was collected in 15 ml polypropylene tube. These were then transported to laboratory in an icebox and stored at -20°C till estimation of IgG concentration.

Digestion of Colostrum: Before performing actual ELISA, all the colostrum samples were digested with rennet (Sigma, USA). For this, 10 ml of each colostrum sample was taken in 50 ml beaker and heated to 37°C in a water bath. 0.5 ml of 0.5% rennet (250 mg in 50 ml distilled water) was added. After 10 minutes clotted colostrum was mixed with glass rod and then filtered via Whatman filter paper no. 42 (quantitative grade) for overnight. Filtered sample was further used for ELISA. Indirect ELISA was performed with the help of kit (Koma Biotech K3231014) with some modifications.

Results and Discussion

In the present study, IgG concentration in colostrum ranged from 11 to 122.1 mg/ml (Table 1) with a mean of 56.98 ± 5.71 mg/ml. The overall mean colostrum IgG concentration (56.98 mg/ml) recorded in the present investigation was well supported by Agrawal (2015) [2] as 50.44 ± 3.36 mg/ml, Chaudhary (2016) [6] as 51.71 ± 5.99 mg/ml and Dang *et al.* (2009) [8] as 54.0 mg/ml in Murrah buffalo using the same method of estimation of IgG (indirect ELISA). The greater variation observed in colostrum IgG in our buffalo dams is in accordance with Kehoe *et al.* (2011) [10] who also observed a wide range of IgG concentration in colostrum (11 – 221 mg/ml). Similarly, Kehoe *et al.* (2007) [11] has also observed extremely high animal variation in IgG concentration ranging from 11.8–74.2 mg/ml in cows. Our mean colostrum IgG concentration was lower than those published by Zhang *et al.* (2001) [18] in bovines (67.2 mg/ml), however, Butler (1973) has reported 50.0 mg/ml colostrum IgG concentration estimated by radial immune diffusion method.

In the present study, the level of IgG in calf serum ranged from 4.2 to 14.88 mg/ml with a mean of 11.23 ± 0.70 mg/ml. Researchers have reported a wide range of immunoglobulin concentration in newborn calf serum. The variation in results could be due to species differences as well as different methods of estimation.

Table 1: IgG concentration in colostrum of dam and serum of calf

S. No.	Dam Number	IgG levels in colostrum of dam	IgG levels in serum of calf
1	M57	101.59	14.26
2	M167TK	68.16	13.69
3	M121TK	33.27	12.01
4	M156	42.51	9.25
5	M153TK	89.58	14.95
6	M68	46.39	10.24
7	M114TK	15.62	4.2
8	M33	62.15	13.59
9	M137TK	38.13	12.73
10	M105	58.1	4.29
11	M85	11	5.26
12	M123	55.9	10.91
13	M94	69.35	9.82
14	MT122	33.69	14.28
15	MT127	122.1	14.11
16	MT129	78.16	13.59
17	MT177	96.2	10.86
18	MT111	44.83	13.76
19	MT91	51.11	14.22
20	MT190	45.71	14.2
21	MT154	25.67	12.52
22	MT116	58.31	8.37
23	MT144	63.15	7.27
	Mean	56.98 ± 5.71	11.23 ± 0.70

Conclusion

Our results revealed that the level of IgG in dam's colostrum and respective calf serum of buffaloes. Levels of IgG in buffalo colostrum's (first milk) estimated by indirect ELISA ranged from 11 to 122.1 mg/ml with a mean of 56.98 ± 5.71 mg/ml and IgG level in calf serum ranged from 4.2 to 14.88 mg/ml with a mean of 11.23 ± 0.70 mg/ml. For a calf to be healthy immunoglobulin (IgG) concentration of 10 mg/ml has been suggested. So by analyzing the serum IgG level we can predict the morbidity and mortality of a calf in a farm.

Acknowledgement

The author is thankful to Joint Director, Instructional Dairy

Farm, Dean, College of Veterinary and Animal Sciences and Director Experimental Station for providing the necessary facilities for conducting the experiment.

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