COLOSTRUM AND SERUM PROTEIN LEVELS IN WATER BUFFALOES[1]

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ABSTRACT - Blood serum samples and colostrum of 17 Murrah water buffalo cows (*Bubalus bubalis*) were examined at birth as well as their offspring before the ingestion of colostrum at 1, 3, 6, 12, 24, 48, 72 and 96 hours after birth to determine the failure in the passive transfer of antibodies. The parameters studied included the total protein (TP), albumin (ALB), alphaglobulin (α GLO), beta-globulin (β GLO) and gamma-globulin (γ GLO) concentration in the serum and colostrum through refractometry and biuret method and protein fractions, separated by electrophoresis. At birth the calves presented a hypogammaglobulinemia or agammaglobulinemia followed by a considerable increase in the serum levels of TP and γ GLO from birth until 96 hours after birth (r=0.9278). However by electrophoresis it could be detected six hours after colostrum ingestion and antibody levels thus being comparable to those in the adult animals, 3.06 g/100 ml. Calves which had suckled showing values up to 0.71 g/ml suggest a failure in the passive transfer, γ GLO values between 0.85 and 1.71 g/100 ml suggest a partial failure and levels above 3.06 g/100 ml indicate an appropriate transfer of antibodies present in the colostrum, a situation of extreme importance for the survival of the newborn calf due to the high content (approximately 82%) of γ GLO.

Index terms: immunological status, gammaglobulins, domestic buffalo.

PROTEÍNAS DO COLOSTRO E DO SORO SANGÜÍNEO DE BUBALINOS

RESUMO - Examinaram-se o soro sangüíneo e o colostro de 17 vacas bubalinas da raça Murrah, no momento do parto, bem como de seus bezerros antes da ingestão do colostro e 1, 3, 6, 12, 24, 48, 72 e 96 horas após o nascimento, com o objetivo de determinar alterações na transferência passiva de anticorpos através do colostro. Foram determinadas as concentrações de proteína total (TP), albumina (ALB), alfaglobulina (α GLO), beta-globulina (β GLO) e gamaglobulina (γ GLO) no soro e no colostro, por meio de refratometria, método de biureto e separação das frações protéicas através de eletroforese. Observou-se que ao nascer os bezerros podem apresentar um quadro de hipogamaglobulinemia, seguido de um aumento considerável nos níveis de TP e γ GLO, do nascimento até 96 horas de vida (r=0.9278). Enquanto isso, através de eletroforese foi possível detectar, após seis horas de vida, que os níveis de anticorpos (γ GLO) são comparáveis aos de um animal adulto, 3,06 g/100 ml. O bezerro bubalino, que ingeriu o colostro e mostrou até 0,71 g/100 ml de γ GLO no soro sangüíneo, sugere falha na transferência passiva de anticorpos, enquanto valores de γ GLO entre 0,85 e 1,71 g/ml indicam uma transferência apropriada de anticorpos presentes no colostro, uma condição de extrema importância para a sobrevivência do recém-nascido, por causa do alto conteúdo de aproximadamente 82% de γ GLO.

Termos para indexação: estado imunológico, gamaglobulinas, búfalo doméstico.

INTRODUCTION

The biological basis of resistance to disease among individuals, breeds of species is due to genetical and environmental factors, the major factor being genetical differences in the ability to produce a specific and effective immune response.

Since the newborn water buffalo calf is hypogammaglobulinemic or agammaglobulinemic the epithelio-corial type of placenta in this domestic species Ram & Chandra (1984) does not allow the transfer of antibodies, immunoglobulins

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(Igs) from the mother to the fetus. The colostral Igs are absorved by endocytosis into the epithelial cells of the small intestine and transferred by exocytosis to the intestinal lymphatic vessel passing through the thoracic duct to the blood stream. During the first weeks after birth, the protection from infectious diseases in the water buffalo calf as in other domestic animals relies exclusively on the passive Igs as evidenced by low serum Igs concentrations in sick animals (Boyd 1972, Kulkarni 1982, Kim & Schmidt 1983). All the Igs thus acquired are essential in combating the diseases producing antigenic invasions. Therefore, the immune status of a newborn animal may be related to the circulating Igs levels. Hence, the purpose of this study was to find out the concentration of serum Igs levels in the dam and buffalo calves as well as in the colostrum and the distribution of different protein fractions which are present in the serum and colostrum of water buffalo bred on the Tropical Humid Amazon conditions.

MATERIALS AND METHODS

The study was conducted from January to April, 1988 on seventeen water buffalo cows and their newborn calves belonging to the Experimental Herd of the Brazilian Enterprise of Agricultural Research (EMBRAPA), Belém, Pará, Brazil. The blood samples were taken from each experimental animal before colostrum ingestion at 1, 3, 6, 12, 48, 72 and 96 hours after birth. 10 ml of blood serum were obtained from the jugular vein and separated by centrifugation after coagulation at room temperature between 22-25°C and stored at -180°C until analyzed. From the colostral samples, whey was prepared by the addition of rennet and stored like the serum until analyzed. Total protein (TP) content in the serum was determined by refractometry (Jain 1986) and colostral TP by the biuret method using kits supplied by Doles Reagents, Brazil and determined by spectrophotometry using Spectronic 20D Milton Company, USA. Determination of protein fractions in the colostrum and serum was done by electrophoresis obtained by gel strips of cellulose acetate in tamponade sodium diethylbarbiturate (Veronal TM) 8.4% and pH 8.6 supplied by Sigma Diagnostic, USA and analyzed in a semimicroeletrophoresis system, CDA Diagnose, Brazil, during 30 minutes, 230 volts in a volume of 1 liter and stained by black amid, Sigma Diagnostic, USA, according to Moura et al. (1987), with the results expressed in percentage (%) through the reading by an automatic electrophoresis integrated densitometer, Tecnow, Brazil, and the value obtained in grams per 100 ml (g/%). The statistical analyses were done by conventional steps and simple correlation and regression (Statistical Analysis Systems 1982).

RESULTS AND DISCUSSION

Average values and standard deviations for TP and fractions of ALB, α GLO, β GLO and γ GLO for serum and colostrum of the dams and in the calves serum are given in Table 1 and Fig. 1. It was observed that of the 17 calves studied, five (30%) were born without antibodies whereas 12 (70%) were born with reduced concentration of antibodies when compared with the dam's. Fig. 1 shows that the TP and GLO levels increased from birth until 24 hours after birth when they became similar to those found in the adult animals. Also it was observed that the absorption levels of antibodies in the colostrum decline 24 hours after birth.

By refractometry the TP concentration increased progressively from birth until 24 hours after birth which means an increase of the TP levels and certainly a good indication that transference of Igs occurred. This was verified by a high correlation between TP and γ GLO (r=0.9278). For regression analysis between γ GLO and time after birth, the cubic model through the coefficient determination, as shown in Fig. 2 ($y=0.880084 + 0.307880x - 0.00641026x + 0.000036477x$), ($r=0.6002$) was used to show that maximum levels were observed within 33 hours. Ig serum values up to 0.75/100 ml in calves which had suckled means a failure in the transfer of colostrum. However, values between 0.85 - 1.71 g;100 ml sueggest a partial failure and levels of 3.06 g;100 ml mean a normal transfer of antibodies from the colostrum. After reaching the peak concentration all these serum constituents decline progressively.

The average values for TP, ALB, α GLO, β GLO, γ GLO, in blood serum and colostrum observed in Fig. 1, are in accordance with other previous reports for water buffalo (Alencar Filho

TABLE 1. Average levels of total protein (TP) and other protein fractions in the blood serum of water buffalo calves, dams and colostrum, g/100 ml.

Time	TP[1]	ALB[2]	GLO[3]	GLO[3]	GLO[3]
0	6.20 ± 0.29	3.15 ± 0.39	1.53 ± 0.30	1.04 ± 0.24	0.71 ± 0.77
1	6.47 ± 0.34	3.19 ± 0.43	1.54 ± 0.36	0.97 ± 0.20	0.85 ± 0.65
3	6.84 ± 1.11	2.92 ± 0.65	1.35 ± 0.33	0.96 ± 0.30	1.71 ± 0.94
6	7.93 ± 0.78	2.67 ± 0.42	1.45 ± 0.29	0.95 ± 0.19	3.06 ± 1.12
12	9.05 ± 1.33	2.47 ± 0.39	1.30 ± 0.23	1.13 ± 0.23	4.05 ± 1.50
24	9.70 ± 1.36	2.48 ± 0.33	1.14 ± 0.18	1.16 ± 0.26	4.86 ± 1.44
48	9.37 ± 1.45	2.46 ± 0.33	1.31 ± 0.19	1.21 ± 0.31	4.47 ± 1.38
72	8.72 ± 1.17	2.48 ± 0.34	1.18 ± 0.17	1.28 ± 0.31	3.79 ± 1.22
96	8.36 ± 0.95	2.44 ± 0.33	1.15 ± 0.19	1.26 ± 0.15	3.52 ± 1.05
Dams	8.79 ± 0.82	3.09 ± 0.54	1.32 ± 0.22	1.50 ± 0.26	2.87 ± 0.62
Colos.	13.33 ± 3.43	0.49 ± 0.21	1.05 ± 0.47	0.87 ± 0.55	10.95 ± 3.08

PT = total protein AL = albumin GLO = Alfablobulin GLO = Betaglobulin GLO = Gammaglobulin

1. Refractometry
2. Biuret method
3. Electrophoresis

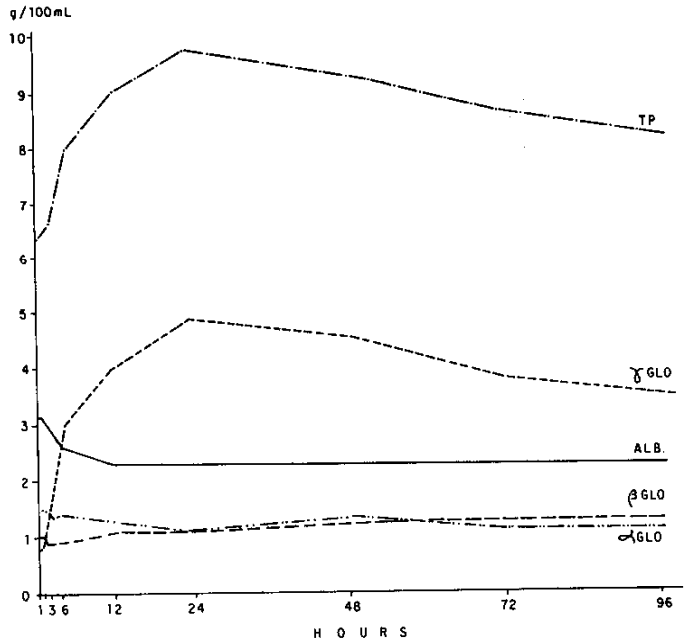

FIG. 1. Average levels of total protein (TP), gammaglobulin (GLO), albumin (ALB), betaglobulin (GLO), and alfaglobulin (GLO) from birth through the following 96 hours.

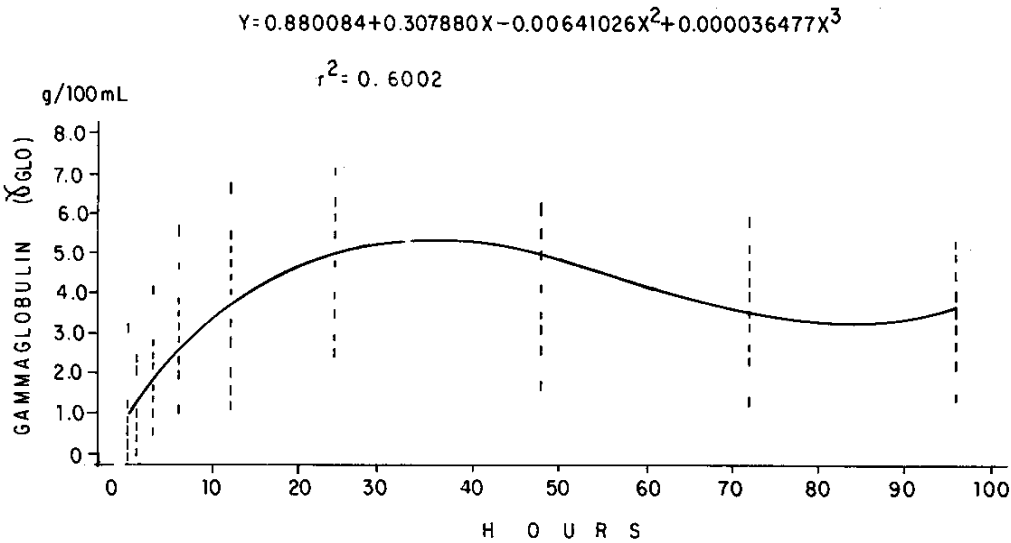

FIG. 2. Regression analysis for the different levels of gammaglobulin (GLO) from birth through the following 96 hours.

1973, Singh et al. 1982, Canfield et al. 1984). However, after birth it was evident that levels of TP and γ GLO increased until 24 hours, (r=0.9278) while they showed a tendency to be the same as in adult animals.

Also it was observed that 5 (30%) of the calves were born without antibodies, whereas 12 (70%) had reduced concentrations when compared with the dams, a phenomenon which agrees with early reports for bovines (Tennant et al. 1969, Bush et al. 1971, Logan & Gibson 1975). In the present study, at the moment of the birth the calves were hypo or agammaglobulinemics, as shown in Fig. 3 and 4, without or with low concentrations of gammaglobulins, which were also reported in bovine species by Tennant et al. (1969), Muller & Ellinger (1981), Logan et al. (1981), Ribeiro et al. (1983). Figure 1 states that the level of antibody absorption declines within 24 hours after birth. This proves that an intestinal block for immune macromolecules from colostrum also occurred in water buffaloes as described for bovines (Kruse 1970, Patt 1977, Donovan et al. 1988).

The total protein levels were used also in other species such as bovine, equine and ovine in order to estimate the antibody concentration. Thus, it is an indirect method used to determine a possible failure of its transference after the calf had ingested the colostrum (Reid & Martinez 1975, Logan & Irwin 1977). Through regression analysis (r=0.6002) as shown in Fig. 2, the γ GLO levels reached their maximum within 33 hours after colostrum ingestion. However, before the first through the third hour there is no maximum peak, although after the sixth hour gammaglobulins are plenty. This certainly means a higher absorption of Igs, a phnomenon also observed in other domestic species, such as the bovine, equine, porcine and ovine (Logan 1978, Kim & Schmidt 1983).

It is evident that after the first hour of life of colostrum ingestion. A substancial increase of GLO ocurred. It continued through the next 24 hours, indicating that the maximum effective absorption occurred within that time, a phenomenon also reported in other species (Patt 1977, Folley &

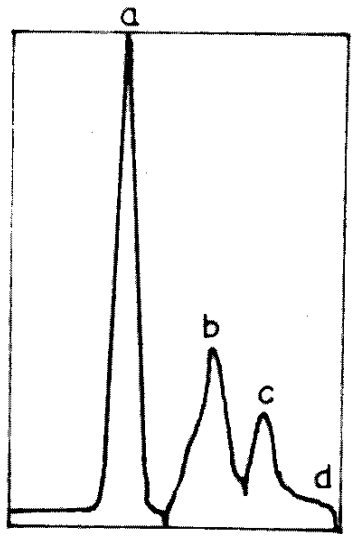

FIG. 3. Eletrophoretic profile of a water buffalo calf hypogammaglobulinemic immediately after the parturition.
a - albumin **c - betaglobulin**
b - alfaglobulin **d - gammaglobulin**

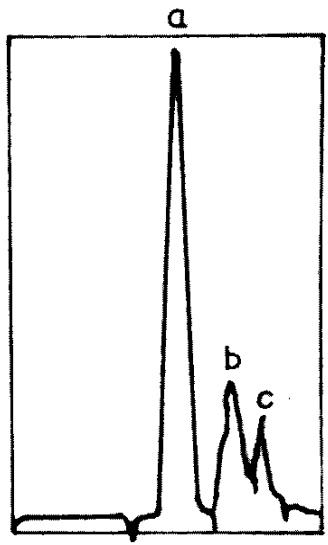

FIG. 4. Eletrophoretic profile of a water buffalo calf agammaglobulinemic immediately after the parturition.
a - albumin **c - betaglobulin**
b - alfaglobulin **d - gammaglobulin**

Otterby 1978, Oyeniyi & Hunter 1978). Furthermore, it is worth considering that after 72 hours a new increase of Igs occurred to a slight diarrhoeic picture which certainly stimuled antibody production.

The high incidence of infections in newborn water buffalo with low serum Igs concentrations substantiates the importance of antibody transfer in this species (Kulkarni 1979, 1982, Láu 1987).

Also for water buffalo it is reasonable that Ig concentration in colostrum used for transfer of maternal immunity to the offspring be of primary importance in assuming a desirable passive immunity.

Values for serum γ GLO up to 0.71 g/100 ml in water buffalo calves health suggest a failure of passive antibody transfer, values between 0.85 and 1.71 g/100 ml suggest a partial failure, and levels higher than 3.06 g/100 ml indicate a passive antibody transference. Herein for normal calves, the serum value for γ GLO showed a variation of 3.06 to 4.86 g/100 ml between 6 to 24 hours after birth, a phenomenon which was also reported for bovines (Tennant et al. 1969, Bush et al. 1971). Detection of low serum GLO levels within six hours after birth would allow the veterinarian to identify newborn water buffalo with high risk for failure of passive antibody transfer and allow an early supplement of colostrum with high contents for these calves.

CONCLUSIONS

1. The determination of TP content can be used as a clinical diagnostic method to detect failure in the antibody transference.

2. The water buffalo calf must suckle the colostrum, within 24 hours after birth since after this there starts the closure of the intestinal lumen to the immuno macromolecules of the colostrum, which contains 82 per cent of γ GLO.

3. The electrophoresis method seems to be one of the best ways to detect Igs; however, it is too expensive to be used routinely in the developing areas of the world.

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