Population Dynamic, Anthelmintic Treatments and the Influence of Helminth Infections on Weight Gain in Water Buffalo Calves (*Bubalus Bubalis*)

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Abstract: The dynamics of gastrointestinal helminthiasis was studied in buffalo calves naturally exposed to helminth infection at two farms in Minas Gerais, Brazil. The effect of helminthiasis was evaluated with respect to weight gain of naturally infected untreated calves versus those which received anthelmintic treatment either with fenbendazole or ivermectin. The treated animals had significantly ($p \leq 0.05$) higher weight gain. *Strongyloides papillosus* was diagnosed from 30 to 60 days after birth, and *Toxocara vitulorum* from 60 to 90 days. During spring, there was a rise in the eggs per gram (EPG) count of Trichostrongylidae. Severe infection of *Paracooperia nodulosa* was detected in few 9-month-old buffalo calves.The results of this study indicate efficacy of fenbendazole and ivermectin in different age group calves for better health and reduced probability of helminth resistance. This study also indicates the necessity for good nutritional management of buffalo calves to improve the efficacy of parasite control.

Keywords: Buffalo, helminthic infection, weight gain.

INTRODUCTION

Buffaloes are an important source of protein for human consumption in many parts of the world. These animals have a higher production efficiency in climates where bovine are not well adapted because for conversion of roughages and fiber into milk and meat [1-3].

Parasitic infestations are responsible for economic losses to buffalo farms because of the unthriftiness, reduced food digestion and absorption and high death rate. The principal parasitic agents in buffaloes are ascarids, fasciolas and paracooperias [4]. In Brazil, the number of buffaloes is increasing and more specific knowledge is needed to control parasitic infestations. Few scientific studies are available about helminth control under Brazilian production conditions to help improve buffalo meat and milk production [5]. Although plenty of literature is available regarding parasitic control in buffaloes, parasitic infestation continue to be a problem in young buffalo calves and result in high mortality rates. Papers published recently by Afridi *et al.* [6] and Khalil-ur-Rehman *et al.* [7] and presentations at the last World Buffalo Congress (Buenos Aires; 2010) indicate that helminth control in buffaloes remains inefficient and ineffective. Therefore, present study was aimed at identifying the dynamics of helminth infection and its importance in the animal's development from birth to 10 months of age, using two different anthelminthics viz. fenbendazole and ivermectin. Fenbandazole and ivermectin are useful to control helminth infection in water buffalo calves if used at correct stage.

MATERIALS AND METHODS

This study was conducted at two typical buffalo milk and meat farms located in the state of Minas Gerais (19°27'46''S 45°36'07' W) with similar production of buffalo milk and meat. No specific protocol was being used to control helminthic infection at these farms. On nearby farms, other breeds of bovine were bred under similar conditions for the production of milk and meat. During the period from January to December 2003, 53 animals of both sexes (23 from farm A / 30 from farm B) of the Jafarabadi breed, ranging from newborns up to 10-months-old, were selected.

The calves on farm A received a diet consisting of 1.5 liters of buffalo milk less a day as compared to those on farm B. After nursing, the dams and calves went to the pasture of *Brachiaria decumbens* until 14:00 hours and then separated into a specific corral and given a supplement of *Pennistum purpureum* and *Zea mays*.

To evaluate the proposed treatments, the animals were divided into six groups, using birth and farm as parameters. On farm A, calves were assigned to group 1 (G1, n=5), group 2 (G2, n=7) and group 3 (G3, n=11). On farm B, there were group 4 (G4, n=10), group 5 (G5, n=11) and group 6 (G6, n=9), as per availability of calves.

Seven days after birth, G1 and G4 animals were treated with 200mcg ivermectin / Kg BW, repeated

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every 60 days until the end of the study period. G2 and G5 calves were treated with 10mg fenbendazole / Kg BW at 15, 30, 60 and 180 days of age, in accordance with a control program recommended by Embrapa Tropicos Umidos – CPATU [8], to control infection by *Toxocara vitulorum*. G3 and G6 animals were kept as untreated controls. The efficacy of therapeutic protocols was analyzed by comparing the results for body weight gain, clinical condition, EPG in feces and the genus of helminth present.

In order to check the tissue lesions resulting from helminth infection and the helminth species involved, an animal from G6 that was 200 days old and positive for genus *Cooperia, Haemonchus, Paracooperia, Oesophagostomum* and *Ostertagia,* was euthanized in accordance with the rules of animal welfare. The intestinal content was placed in formalin solution and the helminth identification analysis was conducted according to the parameters described by Skrjabin [9]. The gastrointestinal areas with macroscopic lesions were collected and fixed on appropriate plates for Hemotoxylin-Eosin staining.

At 30 days' intervals, the body weights of all animals were recorded and feces collected for counting the number of eggs per gram (EPG) and parasite genus identification as per Gordon and Whitlock and Robert and O'Sullivan and Baermann exams (described by Ueno and Mooncalves, [10]. The collected material was transported to the parasitological laboratory at the Federal University of Minas Gerais Veterinary School (Escola de Veterinária da UFMG) under refrigeration with artificial ice in an appropriate container.

For statistical analysis of body weights and EPG counts among the experimental groups and farms, SAS [11] software was used to apply S.N.K test ($p<0.05$). For the non-parametric variables, the Friedman and Kruskal-Wallis ($p<0.05$) test was used. The efficacy of treatments was analyzed by comparing EPG counts using the animal age and treatment as variable parameters.

RESULTS

The EPG count for each group is given in Tables according to the group, age, farm and the parasite species. The data of EPG for the species *Toxocara vitulorum*, *Strongyloides papillosus* and for the helminthes family Trichostrongylidae are present in Tables **2**, **3**, and **4**, respectively.

The genus *Toxocara sp.*, *Strongyloides sp.*, *Cooperia sp.*, *Haemonchus sp.*, *Ostertagia sp.* and *Oesophagostomum sp.* were identified in animals from both, the farm A and farm B. Five animals in G6 from farm B were identified harbouring *Paracooperia* genus.

Table 1. Mean Count and Standard Deviation of Helminthes Eggs Per Gram (EPG) of Feces in Buffalo Calves, Bred on Farms A and B at Dôres do Indaiá, MG, at Various Intervals During Early Age, Treated with Ivermectin (G1and G4, 200mcg/kg BW), Fenbendazole (G2 and G5, 10 mg/kg BW) and Untreated (G3 and G6) from January to December 2003 (There is no Mention of Months – you can Simply Say 'Over a Period of One Year')

	Farm. A						Farm. B					
Calf Age (days)	G1 Ivermectin treated		G2 Fenbendazole treated		G3 Untreated controls		G4 Ivermectin treated		G5 Fenbendazole treated		G6 Untreated controls	
	N	EPG	N	EPG	N	EPG	N	EPG	N	EPG	N	EPG
7	2	0	0	-	4	0	2	100.00±141.42	3	1000.00±1732.05	3	33.33±57.73
15	2	0	2	0	3	933.33±1616.58	3	0	4	0	3	0
30	2	0	4	1275.00±602.07	4	2000.00±2423.49	3	0	5	0	5	180.00±349.28
60	4	25±50	7	125.00±353.55	13	3346.15±7621.95	9	700.00±2100.00	11	0	8	5437.50±10789.93
90	5	33.33±81.64	7	71.42±111.26	11	1045.45±1407.38	9	0	11	72.70±210.19	9	344.44±804.84
120	5	40.00±54.77	7	157.14±214.91	9	533.33±800.00	9	0	11	0	8	75.00±116.49
150	4	25±50	7	42.85±113.38	10	100.00±105.40	9	0	9	0	9	11.11±33.33
180	5	0	6	100.00±126,49	10	40.00±96.60	8	12.50±35.35	11	0	9	10.00±31.62
210	4	0	7	14.28±37.79	10	70.00±105.93	10	0	11	0	8	0
240	5	0	7	0	9	44.44±52.70	9	0	9	0	7	0
270	5	20±44.72	4	50.00±100.00	10	60.00±134.98	7	0	11	0	9	190.90±326.96
300	2	80±109.54	7	0	6	16.66±40.82	9	11.11±33.33	11	75.00±200.56	9	55.55±133.33

Table 2: Mean Count and Standard Deviation of *Toxocara vitulorun* Eggs Per Gram (EPG) of Feces in Buffalo Calves Bred on Farms A and B at Dôres do Indaiá, MG, Treated with 200mcg/kg BW of Ivermectin (G1and G4), 10 mg/kg BW of Fenbendazole (G2 and G5) and Untreated (G3 and G6) from January to December 2003

Age (days)	G1		G2		G3		G4		G5		G6	
Farm	A		A		A		B		B		B	
	N	EPG	N	EPG	N	EPG	N	EPG	N	EPG	N	EPG
7	2	0	0	-	4	0	2	100.00±141.42[a]	3	0	3	33.33±57.73[b]
15	2	0	2	0	3	0	3	0	4	0	3	0
30	2	0	4	12.500±250.00	4	0	3	0	5	0	5	180.00±349.28[ab]
60	4	0	7	0	13	676.92±1337.38[a]	9	700.00±2100.00[a]	11	0	8	4512.50±8618.15[a]
90	5	0	7	0	11	300.00±728.01[ab]	9	0	11	9.09±30.15	9	222.22±666.66[ab]
120	5	20.00±44.72	7	0	9	22.22±66.66[b]	9	0	11	0	8	0
150	4	0	7	0	10	0	9	0	9	0	9	0
180	5	0	6	0	10	0	8	0	11	0	9	0
210	4	0	7	0	10	0	10	0	11	0	8	0
240	5	0	7	0	9	0	9	0	9	0	7	0
270	5	0	4	0	10	0	7	0	11	0	9	0
300	2	0	7	0	6	0	9	0	11	0	9	0

Means with different superscripts in a column differ significantly as per Friedman Test ($p<0.05$).

Table 3: Mean Count and Standard Deviation of *Strongyloides papillosus* Eggs Per Gram (EPG) of Feces in Buffalo Calves Bred on Farms A and B at Dôres do Indaiá, MG, Treated with 200mcg/kg BW of Ivermectin (G1 and G4), 10 mg/kg BW of Fenbendazole (G2 and G5) and Untreated (G3 and G6) from January to December 2003

Age*(days)	G1		G2		G3		G4		G5		G6	
Farm	A		A		A		B		B		B	
	N	EPG	N	EPG	N	EPG	N	EPG	N	EPG	N	EPG
7	2	0	0	-	4	0	2	0	3	1000.00±1732.05	3	0
15	2	0	2	0	3	933.33±1616.58	3	0	4	0	3	0
30	2	0	4	1100.00±836.66	4	1250.00±2433.78	3	0	5	0	5	0
60	3	33.33±57.73	7	112.50±318.19	13	2653.84±6481.46	9	0	11	0	8	925.00±2536.44
90	5	33.33±81.64	7	71.42±111.26	11	709.09±826.38	9	0	11	63.63±211.05	9	111.11±242.09
120	5	20.00±44.72	7	157.14±214.91	9	500.00±792.14	9	0	11	0	8	62.50±91.61
150	4	0	7	28.57±75.59	10	90.00±87.55	9	0	9	0	9	0
180	5	0	6	33.33±81.64	10	30.00±94.86	8	12.50±35.35	11	0	10	10.00±31.62
210	4	0	7	0	10	0	10	0	11	0	8	0
240	5	0	7	0	9	11.11±33.33	9	0	9	0	7	0
270	5	0	4	0	10	0	7	0	11	0	10	18.18±60.30
300	5	0	7	0	6	0	9	0	11	0	9	0

*No significant effect for age and farm according to Friedman and Kruskal-Wallis, respectively ($p>0.05$).

The helminthes species *Haemonchus similis, H. contortus, Ostertagia trifurcata, Paracooperia nodulosa, Cooperia punctata and Oesophagostumun radiatum* were identified in the gastrointestinal tract of the euthanized animal; the locations of the helminthes are presented in Table **5**. The final weight of 300-day-old animals in the three groups was statistically different ($p<0.05$) on farms A and B (Table **6**).

DISCUSSION

An important finding to be highlighted is the interaction between several factors that can influence

Table 4: Mean Count and Standard Deviation of Gastrointestinal Nematodes (Order Strongylidea) Eggs Per Gram (EPG) of Feces in Buffalo Calves Bred on Farms A and B at Dôres do Indaiá, MG, Treated with 200mcg/kg BW of Ivermectin (G1 and G4), 10 mg/kg BW of Fenbendazole (G2 and G5) and Untreated (G3 and G6) from January to December 2003

Age (days)	G1		G2		G3		G4		G5		G6	
Farm	A		A		A		B		B		B	
	N	EPG	N	EPG	N	EPG	N	EPG	N	EPG	N	EPG
7	2	0	0	OPG	4	0	2	0	3	0	3	0
15	2	0	2	0	3	0	3	0	4	0	3	0
30	2	0	4	50.00±100.00	4	750.00±1500.00	3	0	5	0	5	0
60	4	0	7	12.50±35.35	13	15.38±37.55	9	0	11	0	8	0
90	5	0	7	0	11	36.36±67.41	9	0	11	0	9	11.11±33.33
120	5	0	7	0	9	11.11±33.33	9	0	11	0	8	12.50±35.35
150	4	25.00±50.00	7	14.28±37.79	10	10.00±31.62	9	0	9	0	9	11.11±33.33
180	5	0	6	66.66±121.10	10	10.00±31.62	8	0	11	0	9	0
210	4	0	7	14.28±37.79	10	70.00±105.93	10	0	11	0	8	0
240	5	0	7	0	9	44.44±52.70	9	0	9	0	7	0
270	5	0	4	50.00±100.00	10	60.00±134.98	7	0	11	0	9	172.72±306.89
300	5	80.00±109.54	7	0	6	16.66±40.82	9	11.11±33.33	11	75.00±200.56	9	55.55±133.33

Table 5: G.I. Tract Location, Numbers and Species of Helminthes Identified in Intestinal Contents of a 200 Days Old Buffalo Calf from G6 Group

Segment of the gastrointestinal tract	Species of Helminth	Number of adult helminthes
Abomasum	Haemonchus similis	7
Abomasum	*H. contortus*	6
Abomasum	*Ostertagia trifurcata*	25
Duodenum	*Paracooperia nodulosa*	93
Duodenum	*Cooperia punctata*	7
Jejunum	*Paracooperia nodulosa*	37
Jejunum	*C. punctata*	9
Íleum	*P. nodulosa*	23
Íleum	*C. punctata*	49
Large Intestine	*Oesophagostumun radiatum*	94
Large Intestine	*O. radiatum*	51

the success of parasitic control measures, such as farm management, individual animal variation and treatment. This reinforces the necessity to adopt good management practices, in parallel to the helminthes control program, according to the genetic make of the herd and individual characteristics of each production system.

A higher EPG was observed at both the farms in April and May as a consequence of the increased frequency of *Toxocara* sp. and *Strongyloides* sp. present in the intestinal tract. According to Starke *et al*. [12], this is a normal phenomenon in neonatal buffalo calves, not treated with antiparasitic drugs, from ingestion of infective larvae present in colostrum and

Table 6: Mean Live Weight (Kg) and Standard Deviation of 300 Days old Buffalo Calves Bred on Farms A and B

Group	Farm A	Group	Farm B
1 – Ivermectin	160.40 ± 26.93^{a}	4 – Ivermectin	187.44±28.15^{a}
2 – Fenbendazole	131.57 ± 27.24^{b}	5 – Fenbendazole	179.33±21.54^{b}
3 – Control	164.00 ±14.22^{a}	6 – Control	170.55±25.14^{c}

Means with different superscripts in a column (within Farm) differ statistically (p<0.05).

milk in the first few days post partum. In October and November, the presence and number of Trichostrongylids increased as a consequence of the *spring-rise phenomenon,* when climatic conditions facilitate larval migration, resulting in pasture and animal infection [13 -15] (Table **4**).

Eggs of *T. vitulorum* were present in feces of buffalo calves between 5 and 120 days of age, but the EPG was significantly (P<0.05) different between treated and untreated calves. Despite the presence of *T. vitulorum* eggs in the feces of 90-day-old calves in the G5, all protocols used were efficient in controlling this helminth. The results on concentration of *T. vitulorum* in feces during the first two months of a calf's life (Table **2**) are according to Connan [16].

The presence of *S. papillosus* was identified between 7 and 270 days of age in buffalo calves in the present study with a higher EPG at 30 and 60 days in G3 and G6, respectively, as previously reported (Starke *et al.* [17-19]. In order to control *S. papillosus* infection, the efficacy of ivermectin (G1 and G4) was greater than fenbendazole (G2 and G5, Table **3**). The elevated number of *S. papillosus* EPG in day 7 was expected in view of the presence of infective larvae in milk after parturition [18].

Regarding the efficacy of protocols to control the trichostrongylid helminthes (Table **4**), the G1, G4 and G5 treatments showed high efficacy, except with the treatment made at day 120 in G1, as indicated by the presence eggs in the feces on day 150 (Table **4**). It is important to highlight the difference observed between G2 and G5, i.e differences between farms with the use of same treatment in the efficacy to control trichostrongylid infection. For G2, the presence of eggs was confirmed at 30, 60, 150, 180, 210 and 270 days of age. These observations prove the low efficacy of the methodology used to control trichostrongylid infection in animals with nutritional restriction and unhygienic environment.

On farm A, the diagnosis of Trichostrongylid larvae was made at 60 days for *Cooperia* and *Haemonchus*, followed by *Oesophagostomum* at 270 days of calf age. On farm B, the diagnosis of Trichostrongylid larvae varied at 150 days for *Cooperia sp*, 240 days for *Oesophagostomum* and 270 days for *Haemonchus* and *Paracooperia*. The variation in these results can be explained by the differences in nutritional management on the farms, where infection with helminthes occurred more frequently with lower daily milk consumption. Trichostrongylid infection is responsible for damage to the gastrointestinal tract and can interfere with digestion and nutritional absorption [20].

In the present study, trichostrongylidae infection was found present in all age groups of buffalo calves and it was possible to quantify a significant (p<0.05) difference in the incidence of trichostrongylidae. After an interruption in the anthelminthic treatment at day 180 in G2, the EPG number of trichostrongylid increased (Table **4**).

P. nodulosa is a specific buffalo helminth and very damaging to the animal [4] (Figure **1**). The diagnosis of parasite *P. nodulosa* and histological analysis of the gut lesion (Figure **2**), with destruction of lamina propria caused by this pathogen [21, 22], proved the importance of adopting specific sanitary practices for buffalo management, as well as the correct diagnosis of the disease.

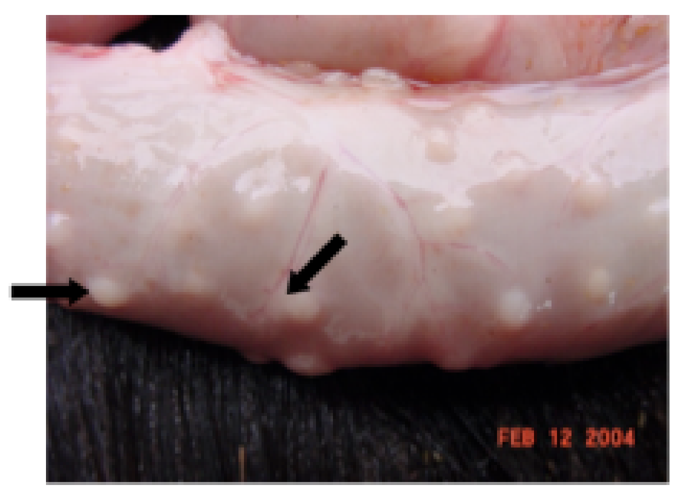

Figure 1: *Paracooperia nodulosa* lesions in intestinal serosa.

The variation in trichostrongylid infection is directly related to the restriction of infective larval development and migration in pasture due to low humidity [14, 15, 23, 24]. As described by other authors, in the present study, the trichostrongylid infection increased following the rains (Table **4** and Figure **3**).

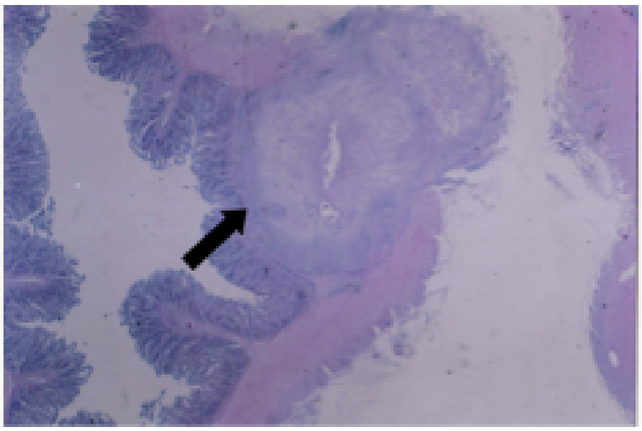

Figure 2: Histological analysis of an intestinal lesion caused by *Paracooperia nodulosa* (H.E., 100x).

Considering the reproductive cycle of buffalo in Brazil [25], the decision to stop the medical intervention at 180 days of age coincides with the beginning of spring, which is not adequate to reduce the impact of trichostrongylid infection. The results of this research indicate that the presence and variation of trichostrongylid eggs in buffalo calves' feces are similar to the other studies in the northern Brazil [26].

The live weights at 300 days age were lower in G1, G2, and G3 than in animals of the same age from G4, G5 and G6 (Table **6**). The body weight growth in calves at farm B was higher, as expected because of the low EPG and better nutritional management. In farm A, the restricted milk allowance for the calves had a negative effect on immunological development to resist helminthes infection while also compromising animal's growth.

The high variation in EPG (Tables **1**, **2**, **3** and **4**) can be attributed to the low sensitivity of the diagnostic test used and less number of animals in the study.

An unexpected result regarding live weights of the animals oat farm A was higher body weight of untreated animals (G3) ($p<0.05$) than the treated animals (G1 and G2). This can be possibly due to the fact that nutritional deficiency interferes with the development of immunological resistance to parasitic infection [27]. A male calf, born on 10/03/2003 died at 115 days with symptoms of severe parasitic infestation. In fecal examination prior to the death of the animal, it was diagnosed for the presence of eggs of S. papillosus at EPG of 2800, 3300, 500 and 200, respectively, at 57, 65, 72 and 94 days of age.

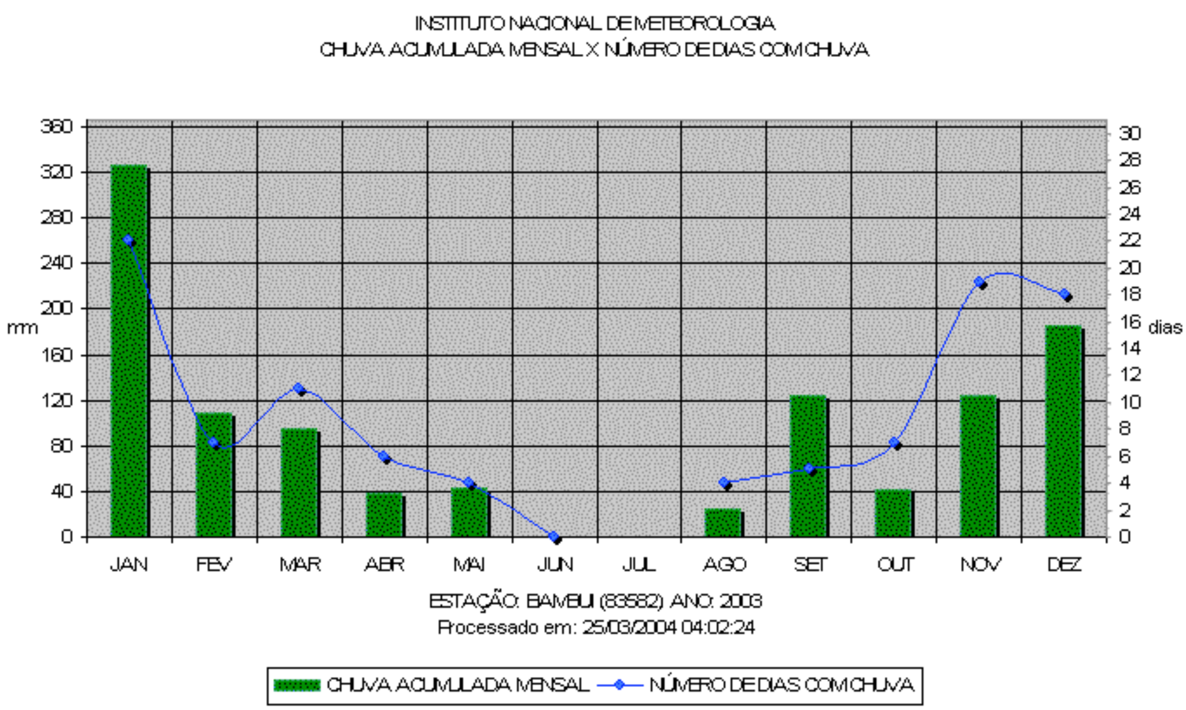

Figure 3: Rainfall distribution in the region during the study period (2003).

CONCLUSIONS

The high EPG frequency of *S. papillosus* and *T. vitulorum* parasites in the feces of buffalo calves from birth to 90 days of age indicates a necessity to treat the calves with specific anti-parasitic drugs within the first week of life, as suggested by [26] and others. Early treatment is necessary to reduce the impact of trichostrongylid infection on the physiological capacity to digest and absorb nutrients, due to damage to gut tissues caused by the formation of permanent lesions. Calves with nutritional deficiencies, such as those resulting from milk restriction without correct supplementation, cannot develop efficient natural resistance to parasites.

In order to program appropriate helminth control in buffalo production system, it is necessary to verify the time of parturition and weaning, as determined by the reproductive cycle, environment and reproductive management in order to control the internal parasites. All the protocols evaluated were efficiently able to reduce helminth infection in buffalo calves and reduce mortality rates, but did not maximize the calf growth, as could be expected using parasite control treatment protocols.

To improve buffalo production through optimum body growth during the first year of life, it is necessary to develop and apply a combination of anti-parasitic drugs in a way that maximizes their efficacy and reduces helminth resistance to the drug. A combination of benzimidazole and ivermectin is useful to combat the most pathogenic water buffaloes parasites - helminthes so as to reduce parasites load and consequent damage to animal body, growth and production.

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