

Natural coccidiosis in water buffaloes (*Bubalus bubalis* L. 1875) in Southeastern Brazil*

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ABSTRACT. Teixeira Filho W.L., Gonçalves L.R. & Lopes C.W.G. **Natural coccidiosis infection in water buffaloes (*Bubalus bubalis* L. 1875) in Southeastern Brazil.** [Coccidiose natural em búfalos (*Bubalus bubalis* L. 1875) no Sudeste do Brasil.] Frequência das espécies do gênero *Eimeria* Schneider, 1885 (Apicomplexa: Eimeriidae) em *Bubalus bubalis* Linnaeus, 1875 no sudeste do Brasil. *Revista Brasileira de Medicina Veterinária*, 38 (supl. 3):1-8, 2016. Departamento de Parasitologia Animal, Anexo 1, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro. Campus Seropédica, BR 465 km 7, RJ 23890-000, Brasil. E-mail: wleira@bol.com.br

Herd of buffaloes in Brazil is related to extensive breedings, where the occupation of the land is premised on its permanence for being a rustic animal. According to the analyzed data obtained from oocysts of the genus *Eimeria* recovered from fecal samples and placed to sporulate in 2.5% potassium dichromate. Regarding the identification of the species, the description and measurements of the sporulated oocysts were taken for each identified species. In relation to the morphotypes found, the presence of three specific species from water buffalo, such as: *Eimeria bareylli*, *Eimeria ankarensis* and *Eimeria gokaki* were observed, where the last two species were first reported in Brazil. The most prevalent species in water buffaloes in the State of Rio de Janeiro was *Eimeria zuernii* followed by *Eimeria ellipsoidal* both and other of less frequent species were also very common in bovine. Species distribution of the genus *Eimeria* was not homogeneous in all analyzed animals, where an animal has eight species, while 88.75% of the total samples had at least four species. The buffalo herds were characterized as extensive breeding, where the animals were kept in place for a long time, regardless of age, and sex and from youth to adults.

KEY WORDS. Water buffaloes, oocysts, *Eimeria*, extensive breedings, Rio de Janeiro, Brazil.

RESUMO. O rebanho de búfalos no Brasil está relacionado a extensões de criação, onde a ocupação da terra se baseia na sua permanência por ser um animal rústico. De acordo com os dados analisados obtidos de oocistos do gênero *Eimeria* recuperados de amostras fecais e colocados para esporular em dicromato de potássio a 2,5%. Quanto à identificação das espécies, foram feitas com base nas descri-

ções e medidas dos oocistos esporulados para cada espécie identificada. Em relação aos morfotipos encontrados, observou-se a presença de três espécies específicas de búfalos de água como *Eimeria bareylli*, *E. ankarensis* e *E. gokaki*, onde as duas últimas espécies foram relatadas pela primeira vez no Brasil. As espécies mais prevalentes em búfalos aquáticos do Estado do Rio de Janeiro foram *E. zuernii* seguidas

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de *E. ellipsoidalis* e outras de menor frequência foram muito frequentes em bovinos. A distribuição de espécies do gênero *Eimeria* não foi homogênea em todos os animais analisados, onde um animal tem oito espécies, enquanto 88,75% das amostras totais tinham pelo menos quatro espécies. Os rebanhos de búfalos foram caracterizados como reprodução extensiva, onde os animais foram mantidos no local por um longo tempo, independentemente da idade, sexo e de jovens para adultos.

PALAVRAS-CHAVE. Búfalos, oocistos, *Eimeria*, criação extensiva, Rio de Janeiro, Brasil.

INTRODUCTION

Herd of buffaloes in Brazil is related to extensive breeding, where the occupation area is premised by being a rustic animal. In this way, the breeds found in the State of Rio de Janeiro present these characteristics.

Coccidiosis is caused by coccidia of the genus *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) and their species are considered as intracellular parasites of the intestinal epithelium cells in the most vertebrates worldwide (Duszynski et al. 2001). Animals become infected by ingesting sporulated oocysts (infective form) through water and food contaminated with feces. The main factor considered in the spread of coccidiosis is persistent contamination of the environment, making the level of infection directly proportional to that of contamination. In this way, the breeding system becomes a factor that has great influence on the level of infection and the characteristics of the eimeriosis. When overpopulation occurs, as in intensive breeding, the disease is more frequent and appears with greater severity, and in buffaloes, eimeriosis is considered to be the cause of higher morbidity and mortality rates in these animals occurring mainly in the first months of life. Severe intestinal lesions. In dairy herds, approximately 70% of the deaths are from lactating animals. This is because neonates, totally devoid of organic resistance, when facing environmental hostilities, become easy targets for pathogens (Baruselli 2000).

Of the *Eimeria* species that affect cattle, two are particularly more pathogenic: *E. bovis* and *E. zuernii* (Ferreira et al. 2002). The species *E. alabamensis*, *E. auburnensis*, *E. bareillyi*, *E. bovis*, *E. brasiliensis*, *E. bukidnonensis*, *E. canadensis*, *E. cylindrica*, *E. ellipsoidalis*, *E. subspherica*, *E. wyomingensis* and *E. zuernii* were also described in buffaloes. With the exception of *E. bareillyi*, all other species are common to cattle and buffaloes (Griffiths 1974, Bhatia 1992).

However, other species may, in certain circumstances or geographic areas, be associated with coccidial infection in buffaloes (Levine 1985).

Silva (1969) reported that eimeriids are the major cause of diseases that affect the growth and development of buffaloes; Griffiths (1974) already emphasized in his studies that eimeriosis was quite common and of sufficient magnitude to cause damage to buffalo calves when these animals were raised in precarious sanitary conditions. This is a fact observed in India and Sri Lanka, where it was to the main cause of mortality of young animals. According to Láu (1990), of the diseases that affect water buffaloes in the Amazon region, the parasitic ones were the most prevalent, where those caused by coccidia was considered one of main factor which interfering in the buffalo rearing in the region.

The susceptibility of the host to these parasites depends on the age, genetic predisposition, innate or acquired immunity, stress level, manipulation, location of the parasite in the intestinal epithelium, and the number of endogenous stages, as well as climatic and other abiotic factors (Hayat et al. 1994).

According to Lima (2004) Among the factors related to coccidia infection that influence the evolution and clinical characteristics of coccidiosis, the *Eimeria* species are associated to the number of cells destroyed by ingested oocysts, which depends on the number of merogonic generations and the number of the merozoites produced by each meront, the infecting dose, the location of the parasite within the tissue cells of the host, the degree of reinfection, the size of the endogenous stages and the viability and virulence of the *Eimeria* species. A sintomatologia pode ser caracterizada por uma infecção aguda resultante de uma enterite associada a fezes líquidas e sangrentas e por intensa inflamação da mucosa intestinal (Griffiths 1974, Sanyal & Ruprah 1984, Láu 1990).

Pavlovic (1975) in the former Yugoslavia reported the species *E. zuernii*, *E. bovis*, *E. cylindrica*, *E. subspherica*, *E. auburnensis*, *E. ellipsoidalis*, and *E. bukidnonensis*, while Sanyal et al. (1985) identified several species as *E. zuernii*, *E. bovis*, *E. cylindrica*, *E. subspherica*, *E. auburnensis*, *E. ellipsoidalis*, *E. bukidnonensis*, *E. bareillyi*, *E. wyomingensis* and *E. canadensis* parasitizing buffaloes in India. In Brazil, buffalo coccidiosis has been reported in several regions, where only the number of species of the genus *Eimeria* found. In the State of Pará, Láu (1982) identified four species as *E. zuernii*, *E. subspherica*, *E. auburnensis* and *E. ellipsoidalis* when Cabral (1987) described the species *E. auburnensis*, *E. canadensis*,

E. cylindrica, *E. ellipsoidalis*, *E. subspherica*, *E. wyomingensis* and *E. zuernii* in buffaloes. The species *E. ellipsoidalis*, *E. zuernii* and *E. wyomingensis* were the most frequent species, being diagnosed throughout the year. *Eimeria ellipsoidalis* predominated in coccidia infection in five months of the study, remained with high indexes in the months of January and February. *Eimeria zuernii* was the most predominant species during four months of the year and *E. wyomingensis* was not predominant at any time.

Barbosa et al. (1992) carried out work to verify natural infection by gastrointestinal parasites in buffaloes reared in the confinement system and outside wetlands in the Botucatu region of São Paulo. They examined 48 buffaloes of the Mediterranean breed, divided into 24 buffaloes and their respective calves. The animals were examined during the first 30 weeks of life. There was 100% positivity for the presence of coccidia in the faeces. Coccidia were observed early in the feces of the calves, because in the third week of life 14 animals eliminated oocysts in their feces (58.33%) and by the sixth week all 24 calves were parasitized.

Hayat et al. (1994) examined samples of bovine and buffalo feces with and without clinical signs of the disease and identified the species: *E. zuernii*, *E. bovis*, *E. cylindrica* and *E. ellipsoidalis*. The mean oocyst size of *E. bovis* was 28.5 × 19.0 µm, of *E. zuernii* 17.85 × 15.25 µm, that of *E. cylindrica* 23.25 × 14.45 µm and that of *E. ellipsoidalis* 17, 0 × 13.5 µm.

Cringolic et al. (1996) with the purpose of investigating the presence and diffusion of *Eimeria* spp. in buffalo herds and the role played by buffalo / postpartum pregnant women in the epidemiology of *Eimeria* spp., Carried out two studies on buffalo breeding farms located in the province of Caserta, in the northwestern part of the Region of Campania (southern Italy). In the first stage of the study 1,184 buffaloes from 130 farms were analyzed: 390 calves, 396 heifers and 398 adult buffaloes. *Eimeria* spp. Was found in 86.1% of the creations examined, where *E. bareillyi*, *E. zuernii*, *E. bovis*, *E. auburnensis*, *E. ellipsoidalis*, *E. subspherica* and *E. pellita* were the most frequently observed species. During the second stage the feces of 80 buffaloes in pregnancy / postpartum were evaluated twice a month, from February to June, in order to find oocysts of *Eimeria* spp. In this group, 76 buffaloes eliminated a limited number of coccidia verified during the months and 32.4% of the exams were positive. The highest number of buffaloes was found in the months of March and April, while the highest numbers of calves occurred in May and June. In the same region,

research was carried out on 180 buffalo farms, between January 1995 and March 1997 by Guarino et al. (2000). In 1620 animals, being 540 buffalo calves, 532 steers / heifers and 548 adults. The survey showed that 25.1% of the animals were positive for species of the genus *Eimeria*, with a higher prevalence among buffalo calves (50.3%), followed by heifers (16.5%) and adults (8.7% %). The species found were: *E. bovis*, *E. bareillyi*, *E. zuernii*, *E. auburnensis*, *E. ellipsoidalis*, *E. subspherica* and *E. pellita*. Two or more species were observed parasitizing the same host. In the same year, Fusgo et al. (1997) carried out a study on a buffalo rearing farm located in southern Italy using 42 calves, in order to evaluate the dynamics of oocyst elimination. Each animal was examined weekly until the 12th week of age, when 100% of the animals eliminated oocysts in the feces. *Eimeria bareillyi* and *E. zuernii* were observed in the second week, *E. ellipsoidalis* and *E. subspherica* (3rd week), *E. auburnensis* (4th week), *E. bovis* (5th week) and *E. pellita* (6th week). No animal presented serious clinical symptoms related to the infection by these coccidia.

In 1997, when examining samples of buffalo feces between 15 days to 12 months of age, born and raised on properties located in the Vale da Ribeira region, 36.4% of the animals tested positive for *Eimeria* species, as *E. zuernii* (10%), *E. bovis* (8.3%), *E. ellipsoidalis* (4.7%), *E. cylindrica* (4.4%) and *E. subspherica* (4.0%), *E. canadensis* (3.1%), *E. auburnensis* (1.5%) and *E. bukidnonensis* (0.3%).

In order to investigate the relationships between the potential agents of diarrhea, passive immunity and occurrence of diarrhea in young buffalo, Ribeiro et al. (2000) worked with the identification of enteropathogens in buffalo calves, with and without diarrhea, from Ribeira Valley, State of São Paulo, Brazil. Parasitological examinations of fecal, diarrheal and non-diarrheal samples of 106 buffalo calves were carried out weekly, for a period of six weeks, 52 males and 54 females, with 3 to 45 days of age of Murrah buffalo calves. The study was conducted from March to July 1996. The prevalence and incidence of diarrhea were observed during the first six weeks of age. Forty-eight calves presented diarrhea and of these, 10 presented enteric signs in two weeks or three throughout the study (totaling 62 diarrheic samples). In the 106 animals examined during the study were identified: *Eimeria* spp., *Strongyloides papillosus*, *Toxocara vitulorum*, *Cryptosporidium parvum*, *Giardia* spp. and representatives of the Strongyloidea Superfamily. The *Eimeria* genus was most frequently identified in all

animals with and without diarrhea, at least in one sample during the study and with its highest prevalence at the age of three weeks. The species identified were: *E. bovis*, *E. zuernii*, *E. auburnensis*, *E. canadensis*, *E. cylindrica*, *E. subspherica* and *Entamoeba coli*. Several associations of bacterial and parasitic agents of origin in the animals with diarrhea were observed. The most frequent associations were *Eimeria* spp., *S. papillosus*, *T. vitulorum* and *E. coli* (12.5%), *Eimeria* spp., *S. papillosus*, *T. vitulorum* and *Enterobacter cloacae* (10.4%). The most frequently observed pathogen in the four studied properties were *Eimeria* spp. and they were the same species found by Rebouças et al. (1990), in the same region, and by Láu (1982) in northern Brazil.

In the review work on eimeriosis in buffalo conducted by Noronha Jr. & Buzetti (2002), *E. ellipsoidalis* species whose sporulated oocysts measured 18-26x13-18µm (mean of 21x15µm) were found, the sporocysts measured 11-16x5-6µm (Average of 12x5µm); *E. zuernii* with oocysts measuring 15-29 x 12-20µm (mean of 20 x 15µm) and sporocysts with 7-10 x 4 -7µm (mean of 9x5µm); *E. auburnensis* with oocysts measuring 32-46x19-28µm (mean of 35x22µm), sporocysts with 16-23x7-11µm (mean of 17x10µm); *E. brasiliensis* with oocysts measuring 31-44 x 20-29µm (mean of 39 x 27µm), sporocysts measuring 16-22 x 8-12µm (mean of 17 x 10µm). In terms of epidemiology, diagnosis, clinical practice, pathology, treatment and prevention of the disease, the authors observed the species of the genus *Eimeria* that parasitize buffaloes, as well as established the presence of pathogenic species and their frequencies, in addition to monitoring the evolution of parasitism in calves and adult buffaloes in two years (one lot per year). They analyzed feces from two lots of 18 buffalo calves from birth to 365 days of age, of both sexes and their respective mothers, from the Unesp/Ilha Solteira Teaching and Research Farm (FEP) in the municipality of Selvíria, MS. The characterization of the species found was made based on the morphometric characteristics. It was observed in both analyzed year, that the highest parasitism index occurred during the rainy season, from September to January in the two years (2000 and 2001). The species identified were *E. ellipsoidalis*, *E. zuernii*, *E. auburnensis*, *E. brasiliensis*, *E. alabamensis* and *E. cylindrica*. The most prevalent were *E. ellipsoidalis* and *E. cylindrica*, which predominated in young animals. *Eimeria zuernii*, on the other hand, presented low parasitic index and only in the adult animals.

In the Netherlands, Dubey et al. (2008) obser-

ved oocysts apparently identical to those of *E. bareillyi*, which were found in feces and small intestine sections of a 22-day-old buffalo calf that died. The shape of the oocysts was constantly piriform, and sometimes presented with lateral asymmetry. The measurements of the non-sporulated oocysts were 23.2-29.5 x 16, 5-22µm, with a mean of 27.2 x 19.3µm for DM and DM respectively. Rodrigues et al. (2009) reported for the first time the encounter of *E. bareillyi* in buffalo from the municipality of Rio Claro, microregion of Sul Fluminense Paraíba Valley, RJ. The oocysts of this species are piriform, with a smooth double wall measuring approximately 2,0 2.0µm. 65 sporulated oocysts were measured, with the largest diameter measuring 32.6 ± 27.3 µm and the smallest diameter 19.6 ± 23.8 µm, as a morphometric index of 1.4 µm. Micropyle was present, residue and polar granule absent in the oocyst. Sporocysts had a diameter greater than 18.7 ± 12.5 µm and less than 8.1 ± 5.9 µm, had residual, polar granule, Stieda body and two refractile bodies.

Fecal samples were collected directly from the rectal ampule of 104 buffaloes in the province of Afyon, Turkey, and analyzed in laboratory to identify coccidia species present. Eleven different species of *Eimeria* and one species of *Isospora* were identified in 75% of the animals tested as follows: *E. zuernii* (55.1%), *E. auburnensis* (44.9%), *E. bovis*, *E. allipsoidalis* (28.2%), *E. ankarensis* (16.7%), *E. subspherica* (16.7%), *E. alabamensis* (11.5%), *E. cylindrica*, *E. bareillyi* (5.1%), *E. canadensis* (5.1%), *E. brasiliensis* (3.8%) and *Isospora* spp. (46.2%). It was the first report of *Isospora* spp. in buffaloes (Nalbantoglu et al. 2008).

Prada et al. (2010) carried out work at La Hacienda Bufalera Suiza, in the department of Puerto Nare in Antioquia, located in the middle Magdalena, Colombia, in order to determine variations in the elimination of eggs per gram of feces (OPG), gastrointestinal parasites during the January to December. A total of 150 buffaloes were divided into three groups of 50 animals (animals aged less than 12 months, 13 to 36 months and over 37 months of age). These animals were randomly selected from a total of 4,200 buffaloes. The annual OPG elimination curves of the Order Strongylidae and, *Strongyloides* spp., *Toxocara* spp. and oocysts per gram of feces (OoPG) of the genus *Eimeria* were established. In the group of animals less than 12 months of age, the eggs of the most frequent parasite during the year were those of *Strongyloides* spp. and oocysts of *Eimeria* spp. In the group of animals with 13 to 36

months of age and more than 37 months, the most frequent parasites were Strongylidae Order and *Eimeria*, *Trichuris* spp. Were the least eliminated. Regarding the elimination of *Toxocara* spp. were only found in the group younger than 12 months of age, with a single presentation in January, June, and August.

Therefore, more studies should be done to identify the actual situation of the buffalo and bovine parasite species and their interactions in the extensive or non - extensive breeding in the state of Rio de Janeiro.

MATERIAL AND METHODS

Flock studied

The animals used in this study came from creations located in the state of Rio de Janeiro. With the exception of one of the farms, located at the Municipality of Rio Claro, which had mixed aptitude, meat / milk, the others were extensive breeding, sometimes in wet lands, where calves remained among adults since calving. This situation made it difficult to group the animals in a picket to be evaluated. Of these animals, 55 animals emptied the rectal ampulla due to stress at the time of collection and no further fecal samples were available. Therefore, the stool was mostly obtained from young animals, which despite being docile were easier to handle.

Place of collection and origin of samples

A total of 121 fecal samples were collected to determine the frequency and species of the genus *Eimeria* as buffalo parasites (*Bubalus bubalis*). After direct collection of the rectal ampulla from the animals, these were stored in plastic bags, previously identified, and maintained at refrigeration temperature until arriving at the laboratory.

Laboratory analysis

In the Coccidia and Coccidiosis Laboratory (LCC) - Department of Animal Parasitology, Annex 1, IV/UFRRJ), each sample was examined in order to determine the presence of coccidia oocysts.

Stool examination. Samples composed of fresh feces were used in the centrifuge-flotation technique according to Sheather (1923), modified by Duszynski & Wilber (1997) for the quantification of oocysts per gram of feces (OoPG).

Sporulation of oocysts. The feces were diluted in a 2.5% aqueous solution of potassium dichromate ($K_2Cr_2O_7$) and placed in 1/2 L plastic bottles containing the ratio of 1/6 faeces to 5/6 solution at room temperature. The material was observed daily until it was verified that approximately 80% of the oocysts were sporulated when the process was considered complete.

Oocyst visualization. After sporulation of the oocysts, 1 mL of the sporulated oocyst containing mixture was placed in 50 mL centrifuge tubes and centrifuged for removal of potassium dichromate and sedi-

mentation recovery. The sediment was then subjected to the centrifugal-flotation technique in saturated sugar solution, density 1.20 for 10 minutes at 500x g in Fanen centrifuge (São Paulo) as described by Sheather (1923) and modified by Duszynski & Wilber (1997). After centrifugation, the contents of the conical tube were filled with saturated sugar solution until formation of a meniscus converging on the surface of the tube, over which a 24 x 32 mm cover slip was placed and left for a period of 10 minutes. After this period, the coverslip was carefully removed and deposited on a previously defatted and dried slide and examined in a Carl Zeiss binocular microscope (RFA) with 40 and 100X objective for the purpose of visualizing the morphological structures.

Measurement of oocysts. A number of sporulated and intact oocysts of the various species of the *Eimeria* genus found were measured. A micrometer eyepiece K-15X PZO (Poland) was used, coupled to the Carl Zeiss binocular microscope. The oocysts and sporocysts were measured in greater diameter (DM) and smaller (dm) and their measurements were dimensioned in μm . In addition, the morphometric index (MI), which is the ratio of DM / dm, was calculated for both oocysts and sporocysts.

Identification of sporulated oocysts. The oocysts found in this study were photographed using a binocular Primo Star Zeiss (RFA) microscope with the Axio-Cam Zeiss ERC5s digital camera (RFA). Identification of sporulated oocysts of species of the genus *Eimeria* by their morphological characteristics.

For the identification of oocysts recovered from buffalo feces, the phenotypic characteristics highlighted by Tenter et al. (2002) and the morphological descriptions of the sporulated oocysts indicated by Duszynski & Wilber (1997) that help in the classification of these coccidia. The morphological characteristics described, when necessary, were used to describe the different species found in buffalo feces.

RESULTS AND DISCUSSION

The buffalo herd in the state of Rio de Janeiro, although not the largest in Brazil, is distributed in all mesoregions. Most often serving more as an occupation animal, especially in areas partially flooded and thus created extensively, where the difficulty of handling is due to the fact that the birth of the puppies occurs in the field and free adult animals in the grass without much contact with their caretakers. On the other hand, it can be observed that some properties managed to lead some management practices, where there is a greater access to the animals, both to young people and adults, separately.

Table 1 shows the measurements of oocysts and sporocysts, as well as the morphometric index of the species found in buffalo feces samples, created in the state of Rio de Janeiro. In the present study, 66.11% of the animals were positive for species of

Table 1. Morphometry of sporulated oocysts of species of the genus *Eimeria* from buffalo (*Bubalus bubalis*) in the state of Rio de Janeiro.

Espécies	n ^a	Oocistos (µm)			Esporocistos (µm)	
		Diâmetros		Índice Morfométrico	Diâmetros	
		Maior	Menor		Maior	Menor
<i>E. alabamensis</i>	17	26.54 (24.20-28.60) ^b	19.70 (16.72-21.56)	1.35 (1.25-1.54)	14.53 (11.00-16.72)	6.02 (3.30-7.26)
<i>E. ellipsoidalis</i>	121	30.10 (18.04-44.88)	20.86 (15.18-28.16)	1.44 (1.04-1.59)	15.23 (9.24-21.34)	6.35 (2.27-9.50)
<i>E. subspherica</i>	10	21.52 (11.66-27.72)	18.04 (11.00-25.08)	1.19 (1.06-1.44)	11.86 (6.82-16.06)	4.57 (1.55-7.04)
<i>E. ankarensis</i>	101	41.07 (34.10-46.20)	26.86 (22.66-30.36)	1.53 (1.36-1.91)	26.86 (13.20-30.36)	7.74 (3.96-19.36)
<i>E. bareillyi</i>	71	29.38 (24.64-33.88)	21.30 (18.70-24.86)	1.38 (1.20-1.56)	16.43 (12.54-19.36)	7.18 (5.94-8.14)
<i>E. bovis</i>	25	25.40 (20.90-36.08)	17.56 (15.40-22.00)	1.44 (1.26-1.82)	12.58 (10.12-15.40)	5.25 (2.42-6.38)
<i>E. cylindrica</i>	17	27.14 (23.76-37.18)	19.57 (14.08-25.52)	1.41 (0.97-1.81)	13.70 (8.80-22.66)	5.67 (3.30-8.80)
<i>E. gokaki</i>	50	24.63 (22.22-28.38)	17.62 (14.96-20.68)	1.40 (1.15-1.71)	13.13 (9.46-14.96)	5.99 (3.96-7.70)
<i>E. zuernii</i>	182	17.43 (11.44-31.02)	14.70 (9.46-22.44)	1.18 (1.01-1.55)	11.06 (6.42-18.48)	5.06 (1.89-7.92)
<i>E. auburnensis</i>	144	41.01 (31.46-41.01)	25.10 (20.90-28.60)	1.64 (1.35-1.96)	18.37 (12.32-22.22)	6.34 (2.71-8.58)
<i>E. brasiliensis</i>	101	39.70 (33.44-47.30)	25.48 (20.46-29.70)	1.57 (1.25-1.59)	18.37 (12.76-23.98)	5.97 (2.81-9.02)

^aNúmero de oocistos esporulados mensurados; ^bLimites, inferior e superior, das medidas dos oocistos esporulados de cada espécie encontrada.

the genus *Eimeria*. The males were the most parasitized (33.88%) in relation to the females (32.23%), being smaller in the researched females. Eleven species were identified as follows: *E. alabamensis*, *E. auburnensis*, *E. bovis*, *E. brasiliensis*, *E. cylindrica*, *E. ellipsoidalis*, *E. subspherica*, *E. zuernii*, *E. bareillyi*, *E. ankarensis* and *E. gokaki*.

Several species of the genus *Eimeria* such as *E. alabamensis*, *E. auburnensis*, *E. bovis*, *E. brasiliensis*, *E. cylindrica*, *E. ellipsoidalis*, *E. subspherica* and *E. zuernii* have been considered common to bovines and buffalo, Pathogenic species appeared more frequently in buffalo flocks in the State of Rio de Janeiro, where *E. zuernii* was the most frequent species (51.87%) (Table 2). This situation, also pointed out by Rebouças et al. (1984) in São Carlos among 10 species of the genus *Eimeria* found and by de Noronha Jr. (2002) in Ilha Solteira, both in the State of São Paulo, Láu (1982) with four species described in the state of Pará; as well as, was similar to that observed by Nalbantoglu et al. (2008) in relation to the species found when working with 104 buffalo feces samples in Ayfon province, Turkey, of which 75% were positive, and 11 species of the genus *Eimeria* was parasitized, parasitizing the animals, distributed as follows: *E. zuernii* (55.1%), *E. auburnensis* (44.9%), *E. bovis* (44.9%), *E. ellipsoidalis* (28.2%), *E. ankarensis* and *E. canadensis* (5.1%), *E. alabamensis*

Tabela 2. Frequency of species of the genus *Eimeria* in buffaloes in the state of Rio de Janeiro based on the morphology of sporulated oocysts.

Species ^a	Oocysts numbers ^b		Total
	Male	Female	
<i>E. alabamensis</i>	115 (1.96) ^c	15 (0.59)	130 (1.55)
<i>E. ankarensis</i> ^d	130 (2.22)	35 (1.37)	165 (1.97)
<i>E. auburnensis</i>	420 (7.18)	345 (13.50)	765 (9.10)
<i>E. bareillyi</i> ^d	155 (2.65)	220 (8.61)	375 (4.46)
<i>E. bovis</i>	50 (0.85)	70 (2.74)	120 (1.43)
<i>E. brasiliensis</i>	265 (4.53)	200 (7.83)	465 (5.53)
<i>E. cylindrica</i>	100 (1.71)	65 (2.54)	165 (1.96)
<i>E. ellipsoidalis</i>	1,115 (19.06)	470 (18.40)	1,585 (18.86)
<i>E. gokaki</i> ^d	125 (2.14)	25 (0.98)	150 (1.78)
<i>E. subspherica</i>	70 (1.20)	55 (2.15)	125 (1.49)
<i>E. zuernii</i>	3,305 (56.50)	1,055 (41.29)	4,360 (51.87)
Total	5,850 (69.60)	2,555 (30.40)	8,405 (100)

^a Based on the morphology of sporulated oocysts; ^b OoPG averages; ^c perceptual values; ^d species observed in water buffaloes only.

Table 3. Number of species of the genus *Eimeria* observed in buffalo feces in the State of Rio de Janeiro.

Species numbers	Values ^a	
	Samples	%
1	27	33,75
2	16	20,00
3	12	15,00
4	16	20,00
5	7	8,75
7	1	1,25
8	1	1,25

^a in 80 fecal samples

(11.55%), *E. cylindrica* (10.3%), *E. bareillyi* (5.1%), *E. brasiliensis* (3.8%). However, they did not find oocysts of *E. gokaki*, observed in this study. *Eimeria canadensis* has not been reported to date in buffalo in the state of Rio de Janeiro.

The specific species of buffalo found in the state of Rio de Janeiro were *E. ankarensis*, *E. gokaki* and *E. bareillyi*, (Table 1). Since *E. ankarensis* and *E. gokaki* were first reported in Brazil, only *E. bareillyi* was previously reported in Brazil by Noronha Jr (2002), Bastianetto et al. (2008), Ramirez et al. (2009) and Meireles et al. (2012). The percentage observed in the total OoPG of the parasitic species of buffaloes, males and females, can be visualized in Table 2. In the same way the relation of species of the genus *Eimeria* according to the specificity in front of the buffalo host is observed, where *E. ankarensis* was the most prevalent with 8.26%, followed by *E. bareillyi* with 3.31% and *E. gokaki* with 2.48% of the parasitized animals. *Eimeria bareillyi*, despite being the most pathogenic (Bastianetto et al., 2008, Meireles et al., 2012) was not the one with the greatest distribution among the number of samples examined in this study. In addition, the species frequently observed in cattle were the most commonly found in buffalo, with *E. zuernii* (51.87%) highly pathogenic for cattle (Soulsby 1987), indicating that there was an alternation in the state of Rio de Janeiro Of bovines and buffaloes or those of bovines replaced by buffaloes, since most species found in buffalo were similar to those observed in cattle in the state of Rio de Janeiro by de Figueiredo et al. (1984).

The number of species of the genus *Eimeria* was not constant in the fecal samples examined, as well as a larger percentage of animals parasitized by a single species, also a lower percentage can be observed, where one animal was parasitized by eight species of the genus *Eimeria* (Table 3). A number of 27 (33.75%) samples were only positive for a single species, but most of them were *E. ellypsoidalis* and *E. zuernii*, and a single sample had eight species, consisting of *E. alabamensis*, *E. auburnensis*, *E. ankarensis*, *E. brasiliensis*, *E. cylindrica*, *E. ellipsoidalis*, *E. subspherica* and *E. zuernii*. This situation was observed only in prairie buffalo (*Syncerus caffer*) in the Kruger National Park in South Africa by Penzhorn (2000), where it also verified that the frequency of species of *Eimeria* was more pronounced and one of the animals and predominantly animals young.

According to the data obtained in the present study, it can be concluded that: in relation to the morphotypes found the presence of three specific

species of buffalo such as *E. bareillyi*, *E. ankarensis* and *E. gokaki*, the last two species were first reported in Brazil. The most prevalent species in the state of Rio de Janeiro was *E. zuernii*. The distribution of species of the genus *Eimeria* was not homogeneous, where one of the samples evaluated was positive for eight species of the genus *Eimeria*, this may be associated with extensive breeding properties, where To the permanence of the animals, regardless of age and sex persists for a long time. With this result it can be affirmed that buffaloes raised extensively in areas traditionally cattle breeding, with the purpose of occupation of the land and some cases used as butcher animals.

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REFERENCES

- ABCB. Associação Brasileira de Criadores de Búfalos. Disponível at: <http://www.bufalo.com.br>. Accessed on: Sept 24, 2012.
- Aumont G., Yvone P. & Esnault A. Experimental coccidiosis in goats. 1. Experimental model effects of parasitism on the feeding behaviour and the growth of animals and intestinal lesions. *Annales de Recherches Vétérinaires*, 15: 467-473, 1984.
- Barbosa M.A., Blasi A.C., Oliveira M.R. & Correa F.M.A. Parasitismo natural de búfalinos em Botucatu, SP, Brasil: III. Dinâmica do parasitismo gastro-intestinal em vacas e suas crias. *Memórias do Instituto Oswaldo Cruz*, 87(supl.1): 37-41, 1992.
- Baruselli P.S. Biotécnicas da Reprodução em Búfalinos. *Arquivos da Faculdade de Veterinária da Universidade Federal do Rio Grande do Sul*, 28(supl. 1):104-157 2000.
- Bastianetto E., Filho E.J.F., Lana A.M.Q., Cunha A.P., Teixeira L.V., Bello A.C.P.P., Teixeira C. & Leite R.C. Epidemiology of *Eimeria* sp. Infection in buffaloes (*Buballus bubalis*) bred in Minas Gerais, Brazil. *Italian Journal of Animal Science*, 6(supl. 2): 911-914, 2007.
- Bastianetto E., Freitas C.M.; Bello A.C., Cunha A.P., Dalla Rosa R.C. & Leite R.C. Primeira notificação de *Eimeria bareillyi* (Apicomplexa: Eimeridae) nas fezes de bezerros búfalos (*Buballus bubalis*) naturalmente infectado em Minas Gerais, Brazil. *Revista Brasileira de Parasitologia Veterinária*, 17(supl. 1):234-238. 2008.
- Bhatia B.B., Pande B.P., Chauhan P.P.S. & Arora G.S. A study on the sporulated oocysts of twelve eimerian species in Indian buffalo (*Buballus buballis*). *Acta Veterinariae Academiae Scientiae Hungarica*, 18 115-133, 1969.
- Bhatia B B. Parasites of river buffaloes, p 309-310. In: Tulloh N.M. & Holmes J.H.G. (Eds), *Buffalo Production*. Amsterdam, FAO, 1992, Cap. 15.
- Chryssafidis A.L., Soares R.M., Rodrigues A.A., Carvalho N.A. & Genari S.M. Evidence of congenital transmission of *Neospora caninum* in naturally infected water buffalo (*Buballus buballis*) fetus from Brazil. *Parasitology Research*, 108:741-743, 2011.
- De Meireles G.S., Da Silva N.M.P., Galvão G. da S., Almeida C.R.R. Flausino W. & Lopes C.W.G. Surto de coccidiose em bezerros búfalos (*Buballus buballis*) por *Eimeria bareillyi* Gil et al., 1963 (Apicomplexa: Eimeriidae) - Relato de casos. *Revista Brasileira de Medicina Veterinária*, 34:116-120, 2012.
- De Noronha Jr A.C.F., Starke-Buzetti W.A. & Duszynski D.W. *Eimeria* spp. in Brazilian water buffalo. *Journal of Parasitology*, 95:231-234, 2009.
- Dubey J.P., Wouda W. & Muskens J. Fatal intestinal coccidiosis in a three weeks old buffalo calf (*Bubalus bubalus*). *Journal of Parasitology*, 94:1289-1294, 2008.

- Duszynski D.W. Increase in size of *Eimeria separata* oocysts during patency. *Journal of Parasitology*, 57:948-952, 1971.
- Duszynski D.W., Upton S.J. & Couch L. The coccidia of the world, 1999. Disponível em: <<http://biology.unm.edu/biology/coccidia/home.html>>. Accessed on: Fev 17, 2012.
- Duszynski D.W. & Wilber P.G. A guideline for the preparation of species descriptions in the Eimeriidae. *Journal of Parasitology*, 83:333-336, 1997.
- Duszynski D.W., Couch L. & Upton S.J. Coccidia (Eimeriidae) of Bovidae (excluding Caprinae). Updated: Fev 13, 2001. Disponível at: <<http://www.k-state.edu/parasitology/worldcoccidia/BOVIDAE>>. Accessed on: 26 Fev 2012.
- FAO. Food and Agriculture Organization. Disponível at: <<http://faostat.fao.org>>. Accessed on: Abr 12, 2012.
- Fayer R. Epidemiology of protozoan infection: the Coccidia. *Veterinary Parasitology*, 6:75-103, 1980.
- Fitzgerald P.R. The economic impact of coccidiosis in domestic animals. *Advances in Veterinary Science and Comparative Medicine*, 24:121-143, 1980.
- Griffiths R.B. Parasites and Parasitic Diseases, p.236-275. In: Cockrill W.R. (Ed.), *The Husbandry and Health of Domestic Buffalo*, Roma, FAO 1974, Cap. 10.
- Hayat C.S., Ruknudin A., Hayat B. & Akhtar M. Prevalence of coccidiosis in cattle and buffaloes with emphasis on age, breed, sex, season and management. *Pakistan Veterinary Journal*, 14:214-217, 1994.
- Lau H.D. *Doenças em búfalos no Brasil*. Brasília: Embrapa/SPI, 1999. 202p.
- Lau H.D. *Eimeriídios parasitos de búfalos no estado do Pará, Brasil*. *Boletim de Pesquisa Embrapa/cpatu*, 42:1-11, 1982.
- Leite R.C. & Lima J.D. Fatores sanitários que influenciam na criação de bezerras. *Arquivos da Escola de Veterinária da Universidade Federal de Minas Gerais*, 34:485-492, 1982.
- Levine N.D. *Veterinary Protozoology*. 1ª ed. Ames, Iowa State University Press, 1985. 414 p.
- Levine N.D. & Ivens V. *The coccidian parasites (Protozoa, Sporozoa) of ruminants*. Urbana, University of Illinois Press. 1970. 274p. [Illinois Biological monographs 44]
- Lima J.D. Coccidiose dos ruminantes domésticos. *Revista Brasileira de Parasitologia Veterinária*, 13(Supl. 1): 9-13, 2004.
- Mimioglu, M., Goksu, K. & Sayin F. *Veterinary and Medical Protozoology II*. 2ª ed. Turkish, Ankara Univ Vet Fak Yay, 1969. Cap. 248, p.1129-1144.
- Nalbantoglu S., Sari B., Cicek H. & Karaer Z. Prevalence of Coccidian Species in the Water Buffalo (*Bubalus Bubalis*) in the Province of Afyon, Turkey. *Acta Veterinaria*, 77:111-116, 2008.
- Noronha Junior A.C.F. & Buzetti W.A.S. Eimeriose em búfalos. *Ciências Agrárias e da Saúde*, 2:47-53, 2002.
- Pellérdy L.P. *Coccidia and Coccidiosis*. 2ª ed. Verlag Paul Parey Berlin, Hamburgo, 1974. 959p.
- Penzhorn B.L. Coccidian oocyst and nematode egg counts of free-ranging African buffalo (*Synceus caffer*) in the Kruger National Park, South Africa. *Journal of South African Veterinary Association*, 71:106-108, 2000.
- Ramirez L., Berto B.P., Teixeira Filho W.L., Flausino W., de Meireles G.S., Rodrigues J. da S., Almeida C.R.R. & Lopes C.W.G. *Eimeria bareillyi* from the domestic water buffalo, *Buballus buballis*, in the State of Rio de Janeiro, Brazil. *Revista Brasileira de Medicina Veterinária*, 31:261-264, 2009.
- Rebhun, W.C. Doenças do Gado Leiteiro. *Roca Ltda*, 1(1): 642, 2000.
- Reboucas M.M., Fujii T.U., Amaral V., Santos S.M., Spósito Filha E., Barci L.A.C. & Fujii T. Eimeriídeos parasitas de búfalos (*Buballus buballis* L.) da região do vale do Ribeira, estado de São Paulo, Brasil. *Arquivos do Instituto Biológico*, 57:1-3, 1990.
- Ribeiro M.G. Langoni H., Jerez J. A. Leite D.S. Ferreira F. & Gennari S.M. Identification of enteropatogens from buffalo calves with and without diarrhoea in the Ribeira Valley, State of São Paulo, Brazil. *Brazilian Journal of Veterinary Research and Animal Science*, 37(2), 2000 [eletrônico].
- Sanyal P.K., Ruprah N.S. & Chhabra M.B. Attempted transmission of three species of *Eimeria* Schneider, 1875 of buffalo-calves to cow-calves. *Indian journal of Animal Sciences*, 55:301-304, 1985.
- Sanyal P.K. & Ruprah N.S. Endogenous stages and pathology in *Eimeria zurnii* coccidiosis in buffalo calves. *Sri Lanka Veterinary Journal*, 32:22-25, 1984.
- Sanyal P.K., Ruprah N.S. & Chhabra M.B. Chemotherapeutic efficacy of sulphadimidine, amprolium, halofuginone and chloroquine fosphate in experimental *Eimeria bareillyi* coccidiosis of buffaloes. *Veterinary Parasitology*, 17:117-122, 1985.
- Sayin F. The sporulated oocysts of *Eimeria ankarensis* n. sp. and of other species of *Eimeria* of buffalo in Turkey and transmission of four species of *Eimeria* from buffalo to cow calves. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 15:282-300, 1968.
- Sayin F. The presence of *Eimeria bareillyi* (Gill, Chhabra and Lal, 1963) in buffalo in Turkey. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 20:38-42, 1973.
- Sayin F. The sporulated oocysts of *Eimeria ankarensis* n. sp. and of other species of *Eimeria* from buffalo cow to calves. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 15:282-300, 1969.
- Sheather A.L. The detection of intestinal protozoa and mange parasites by a flotation technique. *Journal of Comparative Pathology*, 36:266-275, 1923.
- Smith B.P. *Tratado de medicina interna de grandes animais*. 2ª ed. São Paulo, Manole, 1993, p.1501-1528.
- Soulsby E.J.L. *Parasitología y enfermedades parasitarias em los animales domésticos*. 2ª Ed., México: Interamericana, 1987. 823p.