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Eimeria Spp. in Brazilian Water Buffalo

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ABSTRACT: *Eimeria* species are frequently found in water buffalo (*Bubalus bubalis*) in Brazil. Here, we report those *Eimeria* spp. that infect buffalos during their first year of life. Fresh fecal samples were examined from 2 groups (1 group/yr for 2 yr, 2000–2002), each with 18 water buffalo calves (both sexes), from birth through 12 mo of age, in Selvíria, MS, Brazil. Five oocyst morphotypes were observed, i.e., *Eimeria ellipsoidalis* and *Eimeria zuernii*, both previously described from water buffalo, and 3 other morphotypes consistent with descriptions of known *Eimeria* spp. from Artiodactyla hosts, but originally described from other genera than those in which we found them (referred to here as *Eimeria* species 1–3). Our results showed that buffalo calves started shedding oocysts in their feces between 6–29 days of age, with the highest concentration ranging from 188–292 oocysts/g of feces. The 3 unnamed oocyst morphotypes in the calf feces resembled *E. auburnensis* (*Eimeria* sp. 3), *E. cylindrica* (*Eimeria* sp. 1), and *E. subspherica* (*Eimeria* sp. 2). The most prevalent species were *Eimeria* sp. 1 and *E. ellipsoidalis*, which dominated in the youngest animals (6 to 133 days old). *Eimeria zuernii* oocysts, in contrast, were found only in low numbers in the feces of older calves (208 to 283 days old). Calves were infected more frequently during the rainy season (September to January) in both years, but cows were negative for *Eimeria* spp., whenever feces were collected (spring, winter, autumn, or summer seasons).

Eimeria Schneider, 1875 (Apicomplexa: Eimeriidae) is a large genus of obligate intracellular parasites of the intestinal epithelium (and other sites) of most vertebrates worldwide. *Eimeria* spp. infect their hosts when water or food contaminated with sporulated oocysts are ingested (Roberts and Janovy, 2005). Eimeriosis in ruminants can cause high mortality and morbidity, especially in calves (Griffiths, 1974; Sanyal and Ruprah, 1984). The susceptibility of hosts to eimerian parasites depends on their age, genetic predisposition, innate or adaptive immunity, stress level, handling, location of the parasite in the intestinal epithelium, and number and location of endogenous stages, as well as climatic and other factors (Hayat et al., 1994).

In Brazil, there are many reports of bovine coccidiosis, but few have documented coccidia in water buffalo (*Bubalus bubalis*). Silva (1969), Costa et al. (1980), Starke et al. (1983), and Láu (1990) reported that eimeriid parasites are the most prevalent and important agents of diseases that affect the growth and development of the water buffalo. Rebouças et al. (1994), also in Brazil, noted that 36% of buffalo calves between 15 days and 12 mo of age were infected with several *Eimeria* spp., and Barbosa et al. (1992) found that all of the 24 calves they examined were infected with these parasites during the first 30 wk of life. The hot and humid Brazilian climate is advantageous to both the expansion of water buffalo herds and the high prevalence of parasites in these animals, particularly younger ones. In experimental infections of buffalo calves with *Eimeria zurnii*, Sanyal and Ruprah (1984) reported diarrhea, anorexia, weakness, and death 25 days after infection.

A herd of about 40 water buffalo (*Bubalus bubalis*) was kept for 12 mo on a 12-ha pasture of *Brachiaria decumbens* grass, with a pond as their water source, located in Selvíria, MS, Brazil, South America (51°24′55.80′′W, 20°22′5.19′′S). The cows were not milked and the calves were kept together in this area with their mothers. From this herd, 18 water buffalo calves, both males and females, were monitored from their birth through 1 yr of age to determine their natural infections with *Eimeria* spp. (2000–2001); this procedure was then repeated for a second group of 18 calves during the second year of our study (2001–2002).

A fecal sample from calves was collected weekly from birth to 6 mo of age and then monthly from the same animals until each was 12 mo old. Feces were taken directly from the rectum of each calf and examined in the laboratory using McMaster chambers (Whitlock, 1948) and expressed as oocysts per gram of feces (OPG). To identify *Eimeria* spp. in adults (n = 40), fecal samples were collected once during the months of January, April, July, and October to represent all the climatic seasons of the year (summer, autumn, winter, and spring).

After quantifying the *Eimeria* oocysts in the feces of each animal, the numbers of OPG were graded from + to +++ as follows: + = <2,500 OPG (weak infection); ++ = 2,550 to 5,000 OPG (moderate infection); and +++ = >5,050 OPG (heavy infection). Fecal samples with >2,550 OPG were placed in Petri dishes containing 2% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) solution, mixed thoroughly, and kept 6–10 days at room temperature to allow sporulation. Feces containing sporulated oocysts were placed into glass bottles with ~100 ml of $K_2Cr_2O_7$ solution and kept at 4 C until they could be processed and identified (as per Duszynski and Wilber, 1997; Duszynski et al., 1999). Fecal samples from animals of the same age groups were pooled into separate bottles. Sporulated oocysts were concentrated using a centrifuge-flotation technique with a concentrated sucrose solution (Sheather, 1923) and then were counted in triplicate using a light microscope (×400), taking 3 samples of 3 ml from each bottle. Oocysts were identified following the guidelines of Duszynski and Wilber (1997). Standardized abbreviations for oocyst/sporocyst structures are those used by Wilber et al. (1998), except that we used SZ instead of SP for sporozoite. Oocyst characters included length (L), width (W), their range and ratio (L/W), micropyle (M), micropyle cap (MC), residuum (OR), and polar granule (PG); sporocyst characters were length (L), width (W), their range and ratio (L/W), Stieda body (SB), substiedabody (SSB), parastieda body (PSB), residuum (SR), sporozoites (SZ), refractile bodies (RB), and nucleus (N) in SZ. Oocysts (n = 20) were measured and photographed using bright field microscopy, and all measurements are in micrometers (μm) with means followed by the ranges in parentheses.

Since the *Eimeria* spp. we saw, or those that they resemble, have been found in a variety of bovid host genera (Bovidae: Bovini), we have used the following abbreviations when the generic names are abbreviated: *Bo* (*Bos*) and *Bu* (*Bubalus*).

The following species were identified in buffalo calves: *E. ellipsoidalis*, *E.* species 1 (=*E. cylindrica*-like), *E.* species 2 (=*E. subspherica*-like), *E.* species 3 (*E. auburnensis*-like), and *E. zuernii*.

Eimeria ellipsoidalis (Fig. 1A) was originally described from *Bo. taurus* and later from several other genera and species of bovids, including water buffalos. Rebouças et al. (1990) reported it in 5% of 24 buffalo calves, ranging from 15 days to 12-mo-old, in Vale do Ribeira, SP, and Láu (1982) reported it and 3 other *Eimeria* species in *Bu. bubalis* in the state of Pará. Here, we found *E. ellipsoidalis* in 7/36 (19%) buffalo calves 14- to 111-days-old (Table I), particularly during the months of March–April (autumn, the beginning of the dry season). The oocysts measured varied slightly in shape from ellipsoidal to slightly ovoidal, each with a thin wall that appeared to be 2-layered. Both M and MC were absent, and oocysts were 21 × 15 (18–26 × 13–18) with a L/W of 1.3. Both OR and PG were absent. Sporocysts were elongate–ellipsoidal, slightly pointed at 1 end, and measured 12 × 5 (11–16 × 5–6) with a L/W of 2.4. Both SR and SB were present, but SSB and PSB were absent. Each SZ had 2 RBs. Concordantly, Levine and Ivens (1970, 1986) made it clear that the oocysts described as *E. ellipsoidalis* by different authors vary considerably in size from 12–32 × 10–29, but with sporocysts that are less variable, i.e., 11–17 × 5–7. This species is considered common in water buffalo and other bovids (Soulsby, 1968).

Oocysts and sporocysts of *Eimeria* species 1 (Fig. 1B) have the following features. Oocyst shape = elongate–ellipsoidal with thin, smooth wall composed of 2 layers; M, 3 wide, appears to be present; MC: absent; L × W: 20 × 15 (16–30 × 12–17); L/W: 1.4; OR, PG: both absent. Sporocyst shape = elongate–ellipsoidal, slightly pointed at 1 end; L × W: 10 × 5 (9–13 × 4–6); L/W: 2.0; SR: present; SB: present; SSB, PSB: both absent. These descriptive parameters resemble those of *E. cylindrica* Wilson, 1931, to some degree. In the original description of *E. cylindrica*, the micropyle (M) was said to be ''unapparent'' and in later descriptions to be absent (Levine and Ivens, 1986), and none

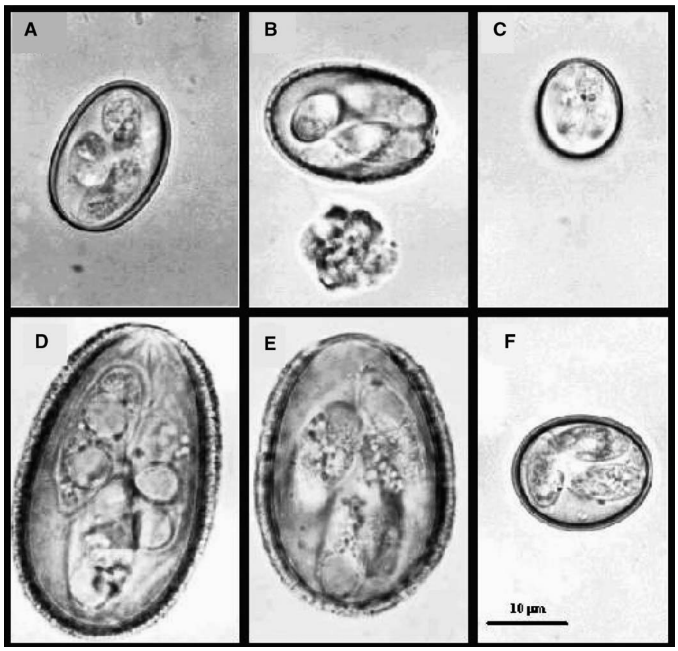

FIGURE 1. Photomicrographs of sporulated oocysts identified in the feces of buffalo calves born in Selviria, MS, Brazil. (**A**) *Eimeria ellipsoidalis*; (**B**) *E.* sp. 1 (*E. cylindrica*-like); (**C**) *E.* sp. 2 (*E. subspherica*-like); (**D, E**) *E.* sp. 3 (*E. auburnensis*-like); and (**F**) *E. zuernii*. Bar = 10 µm.

of the published line drawings of this species shows an M (e.g., Christensen, 1941; Joyner et al., 1966; Levine and Ivens, 1967; Sayin, 1969). The species we observed clearly has an M. Another qualitative difference is that oocysts of *E. cylindrica* are reported to have a PG of many small fragments, which we did not see. Oocysts of *E. cylindrica* were first described from *Bo. taurus* and later reported from *Bo. indicus* and *Bu. babalis*. Levine and Ivens (1970) speculated that the form reported from water buffalo in India may be a separate species from *E. cylindrica* in *Bo. taurus* in the U.S.A. Prior to the present study, Rebouças et al. (1990) reported *E. cylindrica* in 4% of 24 buffalo calves, ranging from 15 days to 12-mo-old, in Vale do Ribeira, SP. Rather than assume the form we saw in *Bu. bubalis* actually is the one that infects *Bo. taurus*, or assume that it is not and call it a new species, we think it best at this time to document its presence in the water buffalo in Brazil and wait until molecular evidence is available to clarify the situation. In the animals we observed, this was the most abundant species in buffalo calves between 6 and 133 days old, being found in 8/36 (22%) calves, particularly during the first 2 mo of age (Table I).

Eimeria species 2 somewhat resembled *E. subspherica* Christensen, 1941 (Fig. 1C). Oocyst shape = subspherical; thin, smooth wall with 2 layers; M, MC: both absent; L × W: 15.5 × 12.7 (11–19 × 10–16); L/W: 1.2 (1.0–1.5); OR, PG: both absent. Sporocyst shape = ovoidal to ellipsoidal, pointed at 1 end; L × W: 6.6 × 2.9 (5–12.5 × 2.5–4); L/W: 2.3 (1.3–3.0); SR: absent; SB: present; SSB, PSB: both absent; 2 RB in SZ. These oocysts differed from the original description in being slightly larger in both range and mean dimensions (11–19 × 10–16 [15.5 × 12.7] vs. 9–14 × 8–13 [12 × 11]), while their sporocysts are similar in size. *Eimeria subspherica* is generally considered to be nonpathogenic under field conditions (Levine and Ivens, 1970). Our *E. subpherica*-like oocysts were present in only 2/36 (7.5%) younger buffalo calves (first 2 mo of age) in small numbers (Table I).

The oocysts and sporocysts of *Eimeria* species 3 (Figs. 1D, E) have the following features. Oocyst shape = elongate–ovoidal; wall with 2 layers, 2.8 (2.5–4.0) thick; outer brown, with rough appearance; M: present, 4.8 (4–6) wide; MC: absent; L × W: 35 × 22 (32–46 × 19–28); L/W: 1.59 (1.3–1.6); OR, PG: both absent. Sporocyst shape = elongate–ellipsoidal, pointed at 1 end; L × W: 12.5 × 5.0 (9–16 × 4–6); L/W 2.5 (2–4); SR: present, often as an irregular mass of globules

TABLE I. Mean numbers of oocysts of 5 *Eimeria* spp. identified in water buffalo (*Bubalus bubalis*) calves of different ages from birth through 12 mo of age, in Selvíria, MS, Brazil during 2 consecutive years (2000, 2001), combined.

		Number of oocysts for each of the 5 *Eimeria* species*				
Buffalo calf no.	Ages (days)	*E. ellipsoidalis* mean ± SD	*E.* sp. 1 mean ± SD	*E.* sp. 2 mean ± SD	*E.* sp. 3 mean ± SD	*E. zuernii* mean ± SD
285	6		2,699.6 ± 130.3			
281	14	563 ± 85.8	2,699.6 ± 130.3			
286	26	319 ± 33				
285	27			496.3 ± 58.7		
287	54	395 ± 78.2				
265	60		1,418.1 ± 136.8			
288	60			76 ± 11.5		
264	61		1,418.1 ± 136.8			
257	94	120.6 ± 7.8	95.6 ± 15.9			
255	99	13 ± 2			25 ± 11.8	
253	100	120.6 ± 7.8	95.6 ± 15.9		162.3 ± 18.6	
249	111	13 ± 2			25 ± 11.8	
282	133		492.6 ± 72.6			
283	147				385 ± 38	
253	172				88.3 ± 17.7	
252	174				88.3 ± 17.7	
257	208					14 ± 1.5
255	212					140 ± 1.5
250	214		1,269.3 ± 110.6			
247	228				1.6 ± 1.1	50.6 ± 7.1
253	283					1.6 ± 1.5

* Oocysts/3 ml of fecal material ± SD (standard deviation).

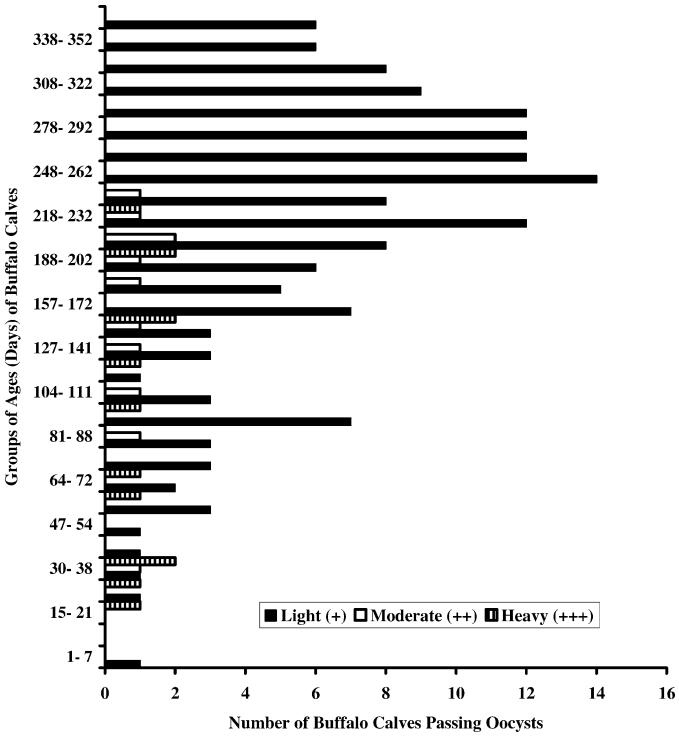

FIGURE 2. Mean numbers of buffalo calves, positive for eimerian oocysts in their feces during the 2 yr of examination (2000, 2001) according to their ages and grade of infection; light (+), moderate (++), and heavy (+++), obtained in Selvíria, MS, Brazil.

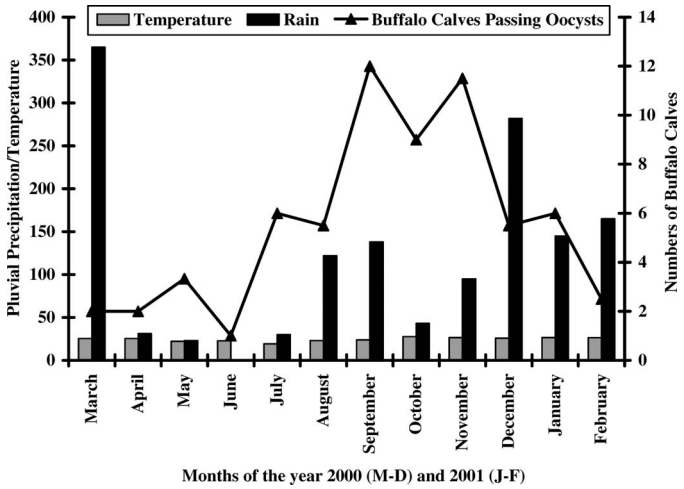

FIGURE 3. Mean numbers of buffalo calves, positive for eimerian oocysts and pluvial precipitation (mm) and temperature (C) of each month (March–December 2000 and January–February 2001), obtained in Selvíria, MS, Brazil.

along one margin of sporocyst; SB: present as a darkened structure at pointed end; SSB, PSB: both absent; SZ each with 2 round RB. These structural features resemble those of *E. auburnensis* Christensen and Porter, 1939, except that our sporocysts were smaller, in both range and mean, than those in previous descriptions (9–16 × 4–6 [12.5 × 5.0] vs. 16–23 × 7–11 [18 or 19 × 8 or 9]). Another potential difference is that our oocysts had a thick, sculptured wall (Figs. 1D, E). Levine and Ivens (1970) said the oocyst wall is ''smooth, rarely rough or heavily mammillated''; however, the original drawing by Christensen and Porter (1939) showed a heavily mammillated outer wall even though they reported finding both smooth, heavily mammillated, and intermediate forms resulting from their infection experiments. Another difference is that we did not see a PG, whereas the original description says a PG is present as 1 large, or many small, scattered fragments. Finally, SZ reported from other hosts described only 1 RB, whereas our SZ clearly have 2 round RB each (Figs. 1D, E). *Eimeria auburnensis* has been reported to infect cattle (*Bo. taurus*) and water buffalo (*Bu. bubalis*), as well as the zebu (*Bo. indicus*), but no cross-transmission work has been done to substantiate this assertion. It is possible that this species is actually *E. auburnensis* and is capable of infecting multiple host genera that are closely related. Because of structural differences noted here, we believe it best at this time to only document its presence in *Bu. bubalis* and wait until molecular data can provide evidence that this is either 1, or multiple, species. This species is known to produce a moderate degree of pathogenicity, including slight diarrhea and apathy, in young calves (*Bo. taurus*). In our study, *E.* sp. 3 was found in 7/36 (19%) older animals, 99- to 122-days-old, but only in small numbers (Table I).

Eimeria zuernii (Fig. 1F) was originally described from *Bo. taurus* and was later reported from *Bo. indicus* (zebu) and *Bu. bubalis* (water buffalo). Sayin (1969) infected 3-wk-old *Bo. taurus* calves with oocysts from water buffalo. Levine and Ivens (1970, 1986) pointed out that the oocysts described as *E. zuernii* by various authors differ considerably in size from 12–29 × 10–21, with means that vary from 17–20 × 14–17. The sporocysts of *E. zuernii* are less variable in size, i.e., 7–13 × 4–7. The oocysts we saw and measured (Fig. 1F) were sub-spheroidal, with a wall ~1 thick composed of 2 layers, outermost being smooth. Both an M and MC are absent. Our oocysts were 20 × 15 (15–29 × 12–20) with L/W: 1.35. OR was absent, but 1 or more PGs were present. Sporocysts were elongate–ovoidal, 9 × 5 (7–10 × 4–7) and L/W, 1.8. A tiny SB is present, but SSB and PSBs are both absent. Each sporocyst had an SR present as tiny globules or granules. This is at least the third time *E. zuernii* has been found in Brazil (Láu, 1982; Rebouças et al., 1990). In this study of natural infections, it was found in 4/36 (11%) older buffalo calves, 208–283 days of age (Table I), and only in the months of October–November (summer season).

Prevalence of eimerian species was analyzed in 2 groups of buffalo calves just after their birth and then following until they reached 1 yr of age in 2000 and 2001 (Fig. 2). Of 18 calves examined in 2000–2001, only 1–3 (6–17%) calves between 22- and 156-days-old had *Eimeria* spp. oocysts in their feces, with a light (+) intensity of infection in the most of them. When the calves were 157- to 367-days-old, 5–14 (28–78%) shed oocysts, but still in small numbers. In 2000, only 1 (6%) and 2 (17%) calves had intensities of ++ or +++; respectively, and they were between 55- and 232-days-old. During 2001, we saw a pattern of infection similar to that in 2000, with only a slightly higher prevalence, and with more infected animals and more intense infections, ranging from moderate (++) to heavy (+++) between 15–247 days of age. In addition, in 2001, 2 calves (11%) had oocysts in their feces earlier, at 7-days-old. The largest number of calves was infected from 157–337 days (5–12 in 2000, 7–16 in 2001). However, because the pattern of infection was similar, the data for the 2 yr were combined (Fig. 2). Barbosa et al. (1992), in São Paulo, found 58% of buffalo calves infected with *Eimeria* spp. during the first 21 days after birth and 100% during the first 30 wk. These authors did not identify which *Eimeria* spp. infected the buffalo they examined, but they suggested that calves could be infected soon after birth, possibly during suckling, by ingesting oocysts adhering to the udder of the cows.

In our herds, most buffalo calves are born in February and March, with a few born as late as April; here, each calf was examined for 1 yr after its birth. The distributions of infected calves/mo are given for 2000–2001 and 2001–2002 (Figs. 3, 4, respectively). For calves born in 2000, 2 peaks of infected calves occurred between September and November (rainy season) when the calves were ~5- to 10-mo-old. However, most calves examined had only light infections (<2,500 OPG). During 2001, the pattern of infection was similar, but a higher number of calves were infected from September to January. From June to October 2001, there was unusually low rainfall (<50 mm), and grass on the pasture was very dry. For that reason, the herd was kept in a smaller area, where the buffalo received supplementary food. This resulted in a higher concentration of animals/m^2 and may have contributed to more environmental contamination with oocysts, thus increasing both their availability and the exposure of animals to infection. On the other hand,

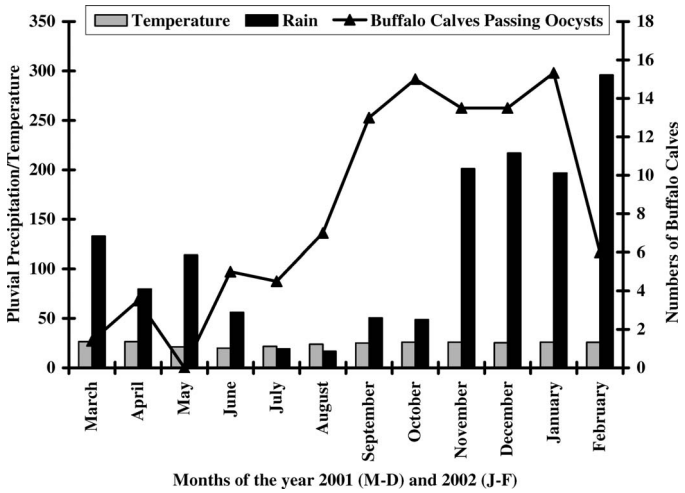

FIGURE 4. Mean numbers of buffalo calves, positive for eimerian oocysts and pluvial precipitation (mm) and temperature (C) of each month (March–December 2001 and January–February 2002), obtained in Selvíria, MS, Brazil.

more rain fell from November to February (>200 mm), and not only did the numbers of infected animals increase, but infection maintained a high plateau until January, before decreasing dramatically (Fig. 4).

According to Hayat et al. (1994), the susceptibility of animals to coccidiosis depends on genetic predisposition, innate or adaptive immunity, stress level, and handling, as well as on climatic and other factors. High temperatures (mean = 25–27 C) and high levels of humidity (110–350 mm pluvial precipitation) occurred in Selviria, MS, during the rainy season (summer) and during the time of our study (Figs. 3, 4). These environmental conditions may have contributed optimal conditions for oocyst sporulation and, thus, for buffalo infection.

In conclusion, buffalo calves from Selvíria, MS, Brazil were found infected with 5 forms of *Eimeria*. Age appears to have some influence on the intensity of the infection, and older calves were more resistant to infection than younger ones. *Eimeria ellipsoidalis* and *E.* sp. 2 were more prevalent in younger calves (6- to 133-days-old), while *E. zuernii* occurred only in low intensity in older calves (208- to 283-days-old). Infected calves did not show any clinical symptoms of coccidiosis. In both 2000 and 2001, higher numbers of animals were found infected during the rainy season (September to January). There was a decrease in the number of infected animals during the month with the lowest pluvial precipitation, suggesting that in the region studied, environmental conditions (particularly rain) can affect oocyst sporulation and pasture contamination. Adult animals did not discharge oocysts in their feces during either year.

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