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Prevalence of *Eimeria* spp. in calves from dairy farms in northern Paraná state, Brazil

Prevalência de Eimeria spp. em bezerros de propriedades leiteiras do norte do estado do Paraná, Brasil

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Abstract

Bovine coccidiosis is a disease of major importance in cattle herds across the world. The disorder mainly affects young calves, and *E. zuernii* are considered the most pathogenic species of the genus, however, *E. alabamensis* have been described in grazing calves. In this study, the prevalence of *Eimeria* spp. was evaluated in calves on dairy farms in the northern region of the state of Paraná, Brazil. Four hundred calves on 44 dairy farms were tested for the presence of coccidian oocysts. The positives were re-examined and the oocysts were morphometrically analyzed for species identification. All the farms were contaminated and 205 animals (51.25%) presented *Eimeria* spp. oocysts. Among these, 146 animals (71.22%) were co-infected by two or more species of coccidia. Ten species of *Eimeria* were identified: *E. bovis* (in 30.25% of the positive samples), *E. alabamensis* (26.75%), *E. zuernii* (22.00%), *E. ellipsoidalis* (18.50%), *E. auburnensis* (13.75%), *E. canadensis* (8.00%), *E. cylindrica* (7.25%), *E. subspherica* (5.00%), *E. bukidnonensis* (3.00%) and *E. brasiliensis* (0.75%). This study demonstrates the high prevalence of *Eimeria* spp. in the northern region of Paraná, Brazil, and detection for the first time in our region the pathogenic species *E. alabamensis*.

Keywords: Coccidiosis, bovine, epidemiology, morphometric, oocysts.

Resumo

A coccidiose bovina é uma doença de grande importância em rebanhos ao redor do mundo. A desordem afeta principalmente bezerros jovens, e *E. bovis* e *E. zuernii* consideradas as espécies mais patogênicas deste gênero, causando grave enterite em animais infectados. No entanto, casos de *E. alabamensis* foram descritos em bezerros mantidos a pasto. No presente estudo, a prevalência de *Eimeria* spp. foi avaliada em bezerros de gado leiteiro da região norte do estado do Paraná, Brasil. Quatrocentos bezerros foram amostrados e testados para a presença de oocistos de coccídios. Os positivos foram re-examinados e os oocistos analisados morfologicamente para identificação da espécie. Todas as fazendas estavam contaminadas e 205 (51,25%) animais apresentaram oocistos de *Eimeria* spp. Destes, 146 (71,22%) animais estava co-infectados por duas ou mais espécies de coccídio. Dez espécies de *Eimeria* foram identificadas: *E. bovis* (30,25% de amostras positivas), *E. alabamensis* (26,75%), *E. zuernii* (22,00%), *E. ellipsoidalis* (18,50%), *E. auburnensis* (13,75%), *E. canadensis* (8,10%), *E. cylindrica* (8,00%), *E. subspherica* (5,00%), *E. bukidnonensis* (3,00%) e *E. brasiliensis* (0,75%). Este estudo demonstra a alta prevalência de *Eimeria* spp. na região norte do estado do Paraná, Brasil, e a detecção, pela primeira vez, de *E. alabamensis*.

Palavras-chave: Coccidiose, bovinos, epidemiologia, morfometria, oocistos.

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Introduction

Eimeriosis is caused by protozoa of the phylum Apicomplexa, family Eimeriidae and genus *Eimeria* (BRUHN et al., 2011). This disease is one of the most common parasitoses in cattle around the world, and it is especially important in animals less than one year old (DAUGSCHIES et al., 2004, BRUHN et al., 2011).

During its life cycle, the parasite destroys the host's enterocytes, causing loss of blood, water, albumin and electrolytes to the intestinal lumen. These effects may lead to diarrhea, dehydration, prostration and eventually death, depending on the period of exposure and infective dose (JONSSON et al., 2011; ALMEIDA et al., 2011).

Among a fourteen known pathogenic species, *E. bovis* and *E. zuernii* are considered to be the most important, due to the severity of clinical cases caused in younger animals (JONSSON et al., 2011; BANGOURA et al., 2011; FLORIÃO et al., 2016). However, some authors have emphasized the importance of *E. alabamensis* as the causative agent of coccidiosis in animals feeding on pastures (SVENSSON et al., 1993, 1994; MARSHALL et al., 1998; SVENSSON, 2000).

This disease is responsible for major economic losses. An impact of US\$ 400 million on the American market has been estimated, due to clinical cases alone, while over US\$ 3.8 million are lost through treatments for bovine coccidiosis in Canada (MATJILA & PENZHORN, 2002; REHMAN et al., 2011).

The rate of occurrence of *Eimeria* spp. is highly variable, with prevalence rates ranging from 10 to 100% among animals tested in Europe and different regions in Brazil (LENTZE et al., 1999, STEWART et al., 2008, ALMEIDA et al., 2011, BRUHN et al., 2011, 2012; KOUTNY et al., 2012). There are just two works concerning about eimeriosis in cattle from south Brazil, both were conducted in Paraná state (GUIMARÁES et al., 1995; HILLESHEIM & FREITAS, 2016), the first one observed 16.7% of Eimeria spp. positivity at northern (GUIMARÁES et al., 1995), and the second one 48.2% of Eimeria spp. positivity at southwestern (HILLESHEIM & FREITAS, 2016). Thus, the objectives of the present study were to determine the prevalence of Eimeria spp. among dairy calves, in herds in the northern region of the state of Paraná, Brazil. This information is relevant because it forms a preliminary stage in devising control schemes against these parasites in dairy herds.

Materials and Methods

Samples

In the present work, a cross-sectional epidemiological study was performed, and four hundred male and female calves, holstein and jersey breed, aged between 20 days and 12 months, on 44 different farms in the northern region of the state of Paraná, Brazil, were sampled. The samples were collected from February 2012 to October 2013. The animals were selected randomly and around 10 animals were included per farm. Feces were collected directly from the rectal ampulla of each animal. All procedures performed in the present study were approved by the Animal Ethics Committee of the State University of Londrina (CEEA/UEL N. 121/2013). The sample size (384) was calculated assuming a prevalence of 50% with a confidence level of 95% and efficacy of 5% by Epi Info 3.5.4. The number of farms was chosen according to convenience, but it fulfilled the minimum sample size that had previously been calculated.

Study area

The climate of northern region of the state of Paraná is characterized as mesothermal with hot summers, infrequent hoar-frosts, and rains with tendency of concentration in the summer months. It presents the following annual averages: temperature of warmer months exceeding 22°C, and of colder months below 18°C; rainfall between 1,300 and 1,700 mm; and a relative humidity of 75%, without water deficiency (IPARDES, 2004). A map with all cities were samples were collected is showed in Figure 1.

Coprological examination and morphological identification

The samples were examined for the presence and number of oocysts per gram of feces (OOPG) using saturated NaCl solution and MacMaster chambers (modified from Gordon & Whitlock, as described in UENO & GONÇALVES, 1998). Positive samples were re-submitted to the flotation technique and oocysts were collected on coverslips, which were observed under an optical microscope for morphological evaluation (modified from Willis & Molay, as described in UENO & GONÇALVES, 1998). Fifty oocysts per sample were analyzed for size, presence of micropyle and wall conformation (REHMAN et al., 2011). The examination was carried out using a B1 microscope (Motic, China), under 400x magnification, with a TK-C138 camera (JVC, Japan) attached to the microscope for documenting *Eimeria* spp. found in the samples (CORNELISSEN et al., 1995; REHMAN et al., 2011; BANGOURA et al., 2011). Eimeria spp. unsporulated oocysts were identified in accordance with parameters described by Levine (1961), and Eckert et al. (1995).

Statistical analysis

The descriptive analysis were performed by the use of Excel software package. For prevalence, and confidence interval at 95% from apparent prevalence OpenEpi version 3.01 was used.

Results and Discussion

Eimeria spp. parasites were widespread and very common in the cattle herds included in this study. From the 400 samples analyzed, 205 (51.25%) were positive to *Eimeria* spp.. Within the positives, 146 (71.22%) were cross infected with multiple *Eimeria* spp. and 59 (28.78%) presented only one species of this parasite genus. The oocyst counts ranged from 50 to 207,800 OOPG and 10 different species were identified by morphological examination (Table 1). The identification of parasitic species, the prevalence of

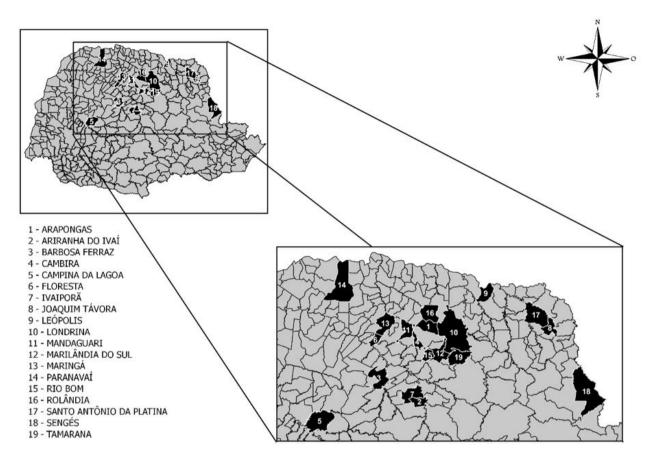


Figure 1. Map of Paraná state, Brazil showing municipalities where calf feces samples were collected in for *Eimeria* spp. occurrence, in the years 2012 and 2013.

Table 1. Identification of <i>Eimeria</i> spp. in dairy calf feces from the northern region of the state of Paraná, Brazil, collected in the years 2012
and 2013. The results of prevalence in samples, farms and their respective morphometry.

Species identified	Nº positive samples (% ^b)	Nº positive farms (% ^b) –	Oocyst Diameter ^a	
			length	width
E. bovis	121 (30.25; 25.95-34.92)	39 (88.64; 76.02-95.05)	27.09 (22.2-33.4)	19.69 (11.4-24.3)
E. alabamensis	107 (26.75; 22.65-31.29)	40 (90.91; 78.84-96.41)	19.72 (15-23.5)	14.73 (10-19.8)
E. zuernii	88 (22.00; 18.22-26.32)	36 (81.82; 68.04-90.49)	18.49 (14.7-23.9)	16.54 (12.5-22.5)
E. ellipsoidalis	74 (18.50; 15.00-22.60)	36 (81.82; 68.04-90.49)	23.31 (19.1-28.3)	15.84 (12.5-19.2)
E. auburnensis	55 (13.75; 10.72-17.47)	30 (68.18; 53.44-80.00)	37.16 (20-45)	23.37 (12.5-29.8)
E. canadensis	32 (8.00; 5.72-11.08)	21 (47.73; 33.76-62.06)	30.64 (26.7-39.6)	21.94 (18.6-26.9)
E. cylindrica	29 (7.25; 5.10-10.22)	18 (40.91; 27.69-55.59)	24.93 (19-35)	13.60 (12-20)
E. subspherica	20 (5.00; 3.26-7.60)	13 (29.55; 18.16-44.22)	12.53 (10-15)	11.75 (9.2-13.75)
E. bukidnonensis	12 (3.00; 1.72-5.17)	9 (20.45; 11.15-34.50)	38.10 (33.2-50)	26.40 (24-28.1)
E. brasiliensis	3 (0.75; 0.26-2.18)	3 (6.82; 2.35-18.22)	37.64 (35.7-40)	26.52 (25-28.2)
Total	400 samples	44 farms		

^aAverage ± standard deviation; ^b(%; CI95%) – CI95% calculated using Wilson score.

infection in animals and farms and morphometric measurements of analyzed specimens are presented in Table 1.

The mean of prevalence in the herds observed here was 56.8% (CI95% = 48.66-64.95). Investigations carried out in different regions of Brazil and around the world have found variable prevalence of *Eimeria* spp. in cattle in dairy and beef herds (CORNELISSEN et al., 1995; CHIBUNDA et al., 1997;

REBOUÇAS et al., 1994; SÁNCHEZ et al., 2008; ALMEIDA et al., 2011; REHMAN et al., 2011; BANGOURA et al., 2011; DONG et al., 2012; ENEMARK et al., 2013). Almeida et al. (2011) found that the prevalence of *Eimeria* spp. contamination among young and adult cattle on dairy farms in the northeast of the state of Bahia, Brazil, was 33.33%. However, the prevalence of this parasite among calves younger than one year of age was

51.22%, which was very similar to the prevalence found in the present study.

One of the problems for the diagnosis of eimeriosis is the fact that it takes several days to sporulated oocysts for photomicrography what is time consuming. Here in the present study, we focused on epidemiological study, by using a faster approach instead time consuming one such as: sporulation and photomicrography. For this, we followed methodology described by Cornelissen et al. (1995), Rehman et al. (2011), Bangoura et al. (2011), and Stewart et al. (2008).

The high frequency of positive samples found in the animals studied here was probably due to the focus on young calves, which is the age group of cattle that is most susceptible to coccidiosis. In dairy herds in Holland, 46% of young calves and 43% of older calves presented infection by *Eimeria* spp., whereas only 16% of cows in the same herds were infected (CORNELISSEN et al. 1995). This suggests that younger animals have higher susceptibility to coccidia, given that clinical cases are almost exclusively seen among animals in this age range (STOCKDALE, 1981; CHIBUNDA et al., 1997; JONSSON et al., 2011; BANGOURA et al., 2011).

Eimeria spp. that are considered to be the most pathogenic to cattle are *E. bovis* and *E. zuernii* (TOMCZUK et al., 2015), which were two of the three most frequent species found in the present study, thus this could suggest that the farms studied here were at high risk of cases of clinical coccidiosis. Both of these species have previously been reported in other states of Brazil, such as São Paulo, Bahia and Minas Gerais (REBOUÇAS et al., 1994; ALMEIDA et al., 2011; BRUHN et al., 2011, 2012). The prevalence of *E. zuernii* ranged from 6.83% to 22.6% in those studies, i.e. very close to the frequency of 22.00% that was found in the present study. The spatial distribution of *E. bovis* in Bahia, where 90% of the farms were contaminated (ALMEIDA et al., 2011), was similar to the distribution of *E. bovis* found in the present study (86.36%).

E. alabamensis has been reported as the causative agent of coccidiosis among grazing calves (SVENSSON et al., 1993, 1994; MARSHALL et al., 1998; SVENSSON, 2000). All over Brazil, including the northern region of the state of Paraná, livestock are fed on pastures more often than in stalls. This may explain why the occurrence of this parasite species among the individuals analyzed was the second highest among all results so far (26.75%), and may also explain the broad distribution across the farms (90.91%). The high prevalence of *E. alabamensis* amongst the animals tested matched the results previously found in Austria, where 45.16% of the animals presented this species (KOUTNY et al., 2012). However, the infection levels found in the present study were much higher than those found elsewhere in Brazil, where the proportion of positive samples ranged from 0.4% to 4.3% among the animals tested and up to 25% of the farms were contaminated (REBOUÇAS et al., 1994; ALMEIDA et al., 2011; BRUHN et al., 2011, 2012; KOUTNY et al., 2012; ENEMARK et al., 2013).

A similar study carried out two decades ago in the same region of the present work found that 16.69% of the animals were infected by *Eimeria* spp. (GUIMARÁES et al. 1995), what it is much lower than the occurrence of 51.25% found in the present study, however, Hillesheim & Freitas (2016) whose worked at southwestern of Paraná, observed 48.2% of samples positives for *Eimeria* spp., this was very similar with prevalence of the present study. *Eimeria alabamensis* was found in 26.75% of the samples of the present study, and 9.1% in the work of Hillesheim & Freitas (2016), but was not described by Guimarães et al. (1995). These differences between our study and Guimarães et al. (1995) work could be associate with the type of samples used, here feces samples were collected among animals without clinical signals, and the other one, most part of the animals (85.5%) had diarrhea.

The broad distribution of *Eimeria* spp., their increasing prevalence and detection for the first time in our region the pathogenic species *E. alabamensis* emphasize the importance of bovine coccidiosis in the northern region of the state of Paraná, Brazil. The epidemiology of coccidiosis, occurrences of clinical cases and evaluations on their economic impact on the respective farms need to be assessed in greater detail. Such studies may provide support for development of a control plan for bovine coccidiosis, which could be extrapolated to other Brazilian regions and states.

References

Almeida VA, Magalháes VCS, Muniz ES Na, Munhoz AD. Frequency of species of the Genus *Eimeria* in naturally infected cattle in Southern Bahia, Northeast Brazil. *Rev Bras Parasitol Vet* 2011; 20(1): 78-81. PMid:21439239. http://dx.doi.org/10.1590/S1984-29612011000100017.

Bangoura B, Mundt HC, Schmäschke R, Westphal B, Daugschies A. Prevalence of *Eimeria bovis* and *Eimeria zuernii* in German Cattle herds and factors influencing oocyst excretion. *Parasitol Res* 2011;109(Suppl 1): 129-138. PMid:21739382. http://dx.doi.org/10.1007/s00436-011-2409-1.

Bruhn FRP, Lopes MA, Demeu FA, Perazza CA, Pedrosa MF, Guimarães AM. Frequency of species of *Eimeria* in females of the Holstein-Friesian breed at the post-weaning stage during autumn and winter. *Rev Bras Parasitol Vet* 2011; 20(4): 303-307. PMid:22166384. http://dx.doi. org/10.1590/S1984-29612011000400008.

Bruhn FRP, Silva FA Jr, Carvalho AHO, Orlando DR, Rocha CMBM, Guimarães AM. Occurrences of *Eimeria* spp. and gastrointestinal nematodes in dairy calves in southern Minas Gerais, Brazil. *Rev Bras Parasitol Vet* 2012; 21(2): 171-175. PMid:22832761. http://dx.doi.org/10.1590/S1984-29612012000200019.

Chibunda RT, Muhairwa AP, Kambarage DM, Mtambo MMA, Kusiluka LJM, Kazwala RR. Eimeriosis in dairy cattle farms in Morogoro municipality of Tanzania. *Prev Vet Med* 1997; 31(3-4): 191-197. PMid:9234443. http://dx.doi.org/10.1016/S0167-5877(96)01131-2.

Cornelissen AWCA, Verstegen R, Van Den Brand H, Perie NM, Eysker M, Lam TJGM, et al. An observational study of *Eimeria* species in housed cattle on Dutch dairy farms. *Vet Parasitol* 1995; 56(1-3): 7-16. PMid:7732653. http://dx.doi.org/10.1016/0304-4017(94)00671-X.

Daugschies A, Imarom S, Ganter M, Bollwahn W. Prevalence of *Eimeria* spp. in sows at piglet-producing farms in Germany. *J Vet Med B Infect Dis Vet Public Health* 2004; 51(3): 135-139. PMid:15107040. http://dx.doi.org/10.1111/j.1439-0450.2004.00734.x.

Dong H, Zhao Q, Han H, Jiang L, Zhu S, Li T, et al. Prevalence of coccidial infection in dairy cattle in Shanghai, China. *J Parasitol* 2012; 98(5): 963-966. PMid:22590990. http://dx.doi.org/10.1645/GE-2966.1.

Eckert J, Taylor M, Catchpole J, Licois D, Coudert P, Bucklar H. Morphological characteristics of oocysts. In: Eckert J, Braun R, Shirley MW, Coudert P. *Biotechnology guidelines on techniques in coccidiosis research*. Luxembourg: European Commission; 1995. p. 103-119.

Enemark HL, Dahl J, Enemark JMD. Eimeriosis in Danish dairy calves – correlation between species, oocyst excretion and diarrhoea. *Parasitol Res* 2013;112(Suppl S1): 169-176. PMid:23765344. http://dx.doi. org/10.1007/s00436-013-3441-0.

Florião MM, Lopes BB, Berto BP, Lopes CWG. New approaches for morphological diagnosis of bovine *Eimeria* species: a study on a subtropical organic dairy farm in Brazil. *Trop Anim Health Prod* 2016; 48(3): 577-584. PMid:26873157. http://dx.doi.org/10.1007/s11250-016-0998-5.

Guimaráes JS Jr, Amaral CHS, Carvalho MCM, Pereira ABL. *Eimeria* spp. (Apicomplexa: Eimeriidae) em bezerros da raça holandesa no norte do Paraná e sudoeste de São Paulo, Brasil. *Rev Bras Parasitol Vet* 1995; 4(2): 71.

Hillesheim LO, Freitas FLC. Ocorrência de eimeriose em bezerros criados em propriedades de agricultura familiar – nota científica. *Ciênc Anim Bras* 2016; 17(3): 472-481. http://dx.doi.org/10.1590/1089-6891v17i333327.

Instituto Paranaense de Desenvolvimento Econômico e Social – IPARDES. *Leituras regionais: Mesorregião Geográfica Norte Central Paranaense*. Curitiba: IPARDES/BRDE; 2004.

Jonsson NN, Piper EK, Gray CP, Deniz A, Constantinoiu CC. Efficacy of toltrazuril 5% suspension against *Eimeria bovis* and *Eimeria zuernii* in Calves and Observations on the Associated Immunopathology. *Parasitol Res* 2011;109(Suppl S1): S113-S128. PMid:21739381. http://dx.doi. org/10.1007/s00436-011-2408-2.

Koutny H, Joachim A, Tichy A, Baumgartner W. Bovine *Eimeria* species in Austria. *Parasitol Res* 2012; 110(5): 1893-1901. PMid:22167365. http://dx.doi.org/10.1007/s00436-011-2715-7.

Lentze T, Hofer D, Gottstein B, Gaillard C, Busato A. Prevalence and importance of endoparasites in calves raised in Swiss cow-calf farms. *Dtsch Tierarztl Wochenschr* 1999; 106(7): 275-281. PMid:10481370.

Levine ND. *Protozoan parasites of domestic animals and of man*. Minneapolis: Burgess Publishing Company; 1961.

Marshall RN, Catchpole J, Green JA, Webster KA. Bovine coccidiosis in calves following turnout. *Vet Rec* 1998; 143(13): 366-367. PMid:9800306. http://dx.doi.org/10.1136/vr.143.13.366. Matjila PT, Penzhorn BL. Occurrence and diversity of bovine coccidia at three localities in South Africa. *Vet Parasitol* 2002; 104(2): 93-102. PMid:11809329. http://dx.doi.org/10.1016/S0304-4017(01)00605-7.

Rebouças MM, Grasso LMPS, Spósito Filha E, Amaral V, Santos SM, Silva DM. Prevalência e distribuição de protozoários do gênero *Eimeria* (Apicomplexa: Eimeriidae) em bovinos nos municípios de Altinópolis, Taquaritinga, São Carlos e Guaíra – Estado de São Paulo, Brasil. *Rev Bras Parasitol Vet* 1994; 3(2): 125-130.

Rehman TU, Khan MN, Sajid MS, Abbas RZ, Arshad M, Iqbal Z, et al. Epidemiology of *Eimeria* and associated risk factors in cattle of district Toba Tek Singh, Pakistan. *Parasitol Res* 2011; 108(5): 1171-1177. PMid:21110042. http://dx.doi.org/10.1007/s00436-010-2159-5.

Sánchez RO, Romero JR, Founroge RD. Dynamics of *Eimeria* oocyst excretion in dairy calves in the Province of Buenos Aires (Argentina), during their first 2 months of age. *Vet Parasitol* 2008; 151(2-4): 133-138. PMid:18096320. http://dx.doi.org/10.1016/j.vetpar.2007.11.003.

Stewart ID, Smith RP, Ellis-Iversen J. *Eimeria* species in cattle on farms in England and Wales. *Vet Rec* 2008; 162(15): 482-483. PMid:18408197. http://dx.doi.org/10.1136/vr.162.15.482.

Stockdale PHG. Effects of monensin on coccidiosis in ruminants. *Vet Med Small Anim Clin* 1981; 76(11): 1575-1578. PMid:6916536.

Svensson C, Hooshmand-Rad P, Pehrson B, Törnquist M, Uggla A. Excretion of *Eimeria* oocysts in calves during their first three weeks after turn-out to pasture. *Acta Vet Scand* 1993; 34(2): 175-182. PMid:8266895.

Svensson C, Uggla A, Pehrson B. *Eimeria alabamensis* infection as a cause of diarrhoea in calves at pasture. *Vet Parasitol* 1994; 53(1-2): 33-43. PMid:8091616. http://dx.doi.org/10.1016/0304-4017(94)90014-0.

Svensson C. Excretion of *Eimeria alabamensis* oocysts in grazing calves and young stock. *J Vet Med B Infect Dis Vet Public Health* 2000; 47(2): 105-110. PMid:10763379. http://dx.doi.org/10.1046/j.1439-0450.2000.00324.x.

Tomczuk K, Grzybek M, Szczepaniak K, Studzińska M, Demkowska-Kutrzepa M, Roczeń-Karczmarz M, et al. Analysis of intrinsic and extrinsic factors influencing the dynamics of bovine *Eimeria* spp. from central– eastern Poland. *Vet Parasitol* 2015; 214(1-2): 22-28. PMid:26455571. http://dx.doi.org/10.1016/j.vetpar.2015.09.027.

Ueno H, Gonçalves PC. Manual para diagnóstico das helmintoses de ruminantes. 4th ed. Tokio: Japan International Cooperation Agency; 1998.