ARTÍCULO

Parasites of Pampas deer (*Ozotoceros bezoarticus* L. 1758) in sympatry with livestock in Uruguayan agroecosystems

Parásitos del venado de las pampas (*Ozotoceros bezoarticus* L. 1758) en simpatría con ganado doméstico en los agroecosistemas uruguayos

Castro Mayra[1], Rosadilla Diego[1], Hernández Zully[1], and González Susana[2]*

ABSTRACT: In the recent past, the Pampas deer (*Ozotoceros bezoarticus* Linnaeus, 1758) has been one of the most characteristic species in Uruguayan grasslands. Currently, the Pampas deer populations are small, endangered and highly isolated, remaining in cattle ranches. The aim of this study is to describe the gastrointestinal parasites of a Pampas deer (*O. b. arerunguaensis*) population that share paddocks and grazing areas with bovine and ovine populations in each season along one year, using coproparasitological techniques. Morphological analysis of eggs and larvae and the quantification of eggs per gram of feces were performed on faecal samples from three ruminant species (119 of deer, 144 of cattle and 85 of sheep) that share three paddocks. The parasitic taxonomic identification was made from infesting larvae obtained by the egg stool culture. Some genera of parasites were shared between domestic ruminants and Pampas deers, in different seasons. The greatest parasite generic richness was found in winter from calves and ovines, being the largest record also observed in the Pampas deers from the paddock C. The epg counting in samples of Pampas deer from the three paddocks showed a similar seasonal behavior throughout the year (p =0.89). We diagnosed the presence of *Haemonchus, Oesophagostomum, Ostertagia*, and *Trichostrongylus* in Pampas deer. Finally, it is recommended to maintain parasitic chemical restraint measures to avoid the transmission of species from domestic ruminants to wild fauna found in sympatry.

Keywords: bovine, ovine, Pampas deer, parasites, Uruguay.

RESUMEN: En el pasado reciente, el venado de las pampas (*Ozotoceros bezoarticus* Linnaeus, 1758) ha sido una de las especies más características del campo uruguayo. Actualmente, sus poblaciones son pequeñas, se encuentran amenazadas y están aisladas en establecimientos agropecuarios. El objetivo de esta investigación es describir la parasitofauna gastrointestinal de una población de venados de las pampas (*Ozotoceros bezoarticus arerunguaensis*) que comparten potreros y áreas de pastoreo con poblaciones de ovinos y bovinos, en las diferentes estaciones a lo largo de un año, usando técnicas coproparasitológicas. Los análisis morfológicos de huevos y larvas y la cuantificación de huevos por gramo de heces fueron realizados sobre muestras fecales de las 3 especies de rumiantes (119 de venados, 144 de bovinos y 85 de ovinos) que comparten tres potreros. La identificación taxonómica parasitaria se realizó a partir de larvas infectantes obtenidas por el cultivo de huevos colectados en las fecas. Algunos géneros de parásitos fueron compartidos entre rumiantes domésticos y venados de las pampas en las diferentes estaciones. La mayor riqueza de géneros fue encontrada en terneros y ovinos en invierno, siendo el mayor registro también observado en el venado del potrero C. El conteo de epg en venados de las pampas de los tres potreros mostró un comportamiento estacional similar a lo largo del año (p = 0.89). En el venado de las pampas se observó la presencia de *Haemonchus*, *Oesophagostomum*, *Ostertagia* y *Trichostrongylus*. Se recomienda la toma de medidas de contención química parasitaria para evitar la transmisión de especies parásitas desde los rumiantes domésticos hacia la fauna silvestre que se encuentre en simpatría.

Palabras clave: bovino, ovino, parásitos, Uruguay, venado de las pampas.

[1] Parasitología, Facultad de Veterinaria Cenur Litoral Norte Salto, Universidad de la República Uruguay. Rivera 1350, CP 50000 Salto, Uruguay.
[2] Departamento Biodiversidad y Genética, Instituto de Investigaciones Biológicas Clemente Estable, Ministerio de Educación y Cultura. Av. Italia 3318, CP 11600 Montevideo, Uruguay.

Correspondencia: sgonzalez@iibce.edu.uy
http://orcid.org/0000-0001-6470-6182

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INTRODUCTION

Formerly, the Pampas deer (*Ozotoceros bezoarticus* L. 1758) occupied a range of open habitats, such as grassland, pampas, savanna, and cerrado, from 5° to 41° south latitude in regions of Argentina, Bolivia, Brazil, Paraguay and throughout the Uruguayan territory (González *et al*., 1998; González *et al.,* 2010, González *et al*., 2016; Fig. 1). The cattle introduction was a key factor for the trophic competition and the breeding promoted the habitat fragmentation and interfered with the Pampas deer dispersal to edge areas (González *et al*., 1998). At the beginning of the 1900, a specific Pampas deer eradication campaign was initiated, similar to the bison situation in North America. This species was persecuted based on the belief that the grassland needs to be exclusively used by the introduced livestock. Furthermore, the appearance of livestock also arrived with new infectious and parasitic diseases (González *et al*., 2010; Hernández and González, 2012).

Currently, the area encompassed by these habitats has been dramatically reduced to less than 1% of that present in 1900 (González *et al*., 1998). The Pampas deer populations are small and highly isolated. In Uruguay, there are only two endemic populations belonging to two subspecies respectively: *O. b. arerunguaensis* González, Álvarez-Valin and Maldonado, 2002 located in the northern part of the country, in Arerunguá Salto (31° 65' S, 56° 43' W), with around 1200 individuals; and *O. b. uruguayensis* González, Álvarez-Valin and Maldonado, 2002 located in the southeast part of the country, in Los Ajos – Rocha (33° 45' S, 54° 02' W) with approximately 300 individuals (González *et al*., 2010; Cosse and González, 2013). Even though Pampas deer has been cataloged by the Uruguayan government as a threatened species, being declared as a living Uruguayan Natural Monument (Ministerial Decree 12/985), management guidelines have not yet been established, neither have any actions been taken for its effective conservation being the populations in private lands (González *et al*., 2010). These populations are only located in private ranches sharing the grasslands with domestic livestock. Both subspecies were declared by the Environmental Ministry as priority for conservation in Uruguay due to the current population decline (Soutullo *et al*., 2013).

Previous studies conducted in Neotropical deer species that inhabit with livestock described the main parasite species that infected them in diverse habitats (Nascimento *et al*., 2000; Duarte *et al*., 2001; Uhart *et al*., 2003; Lux Hoppe *et al*., 2010; Hernández and González, 2012; Orozco *et al*., 2013). These authors described the parasitic fauna richness found in Neotropical deers but did not perform the simultaneous analysis and description in livestock that inhabit in

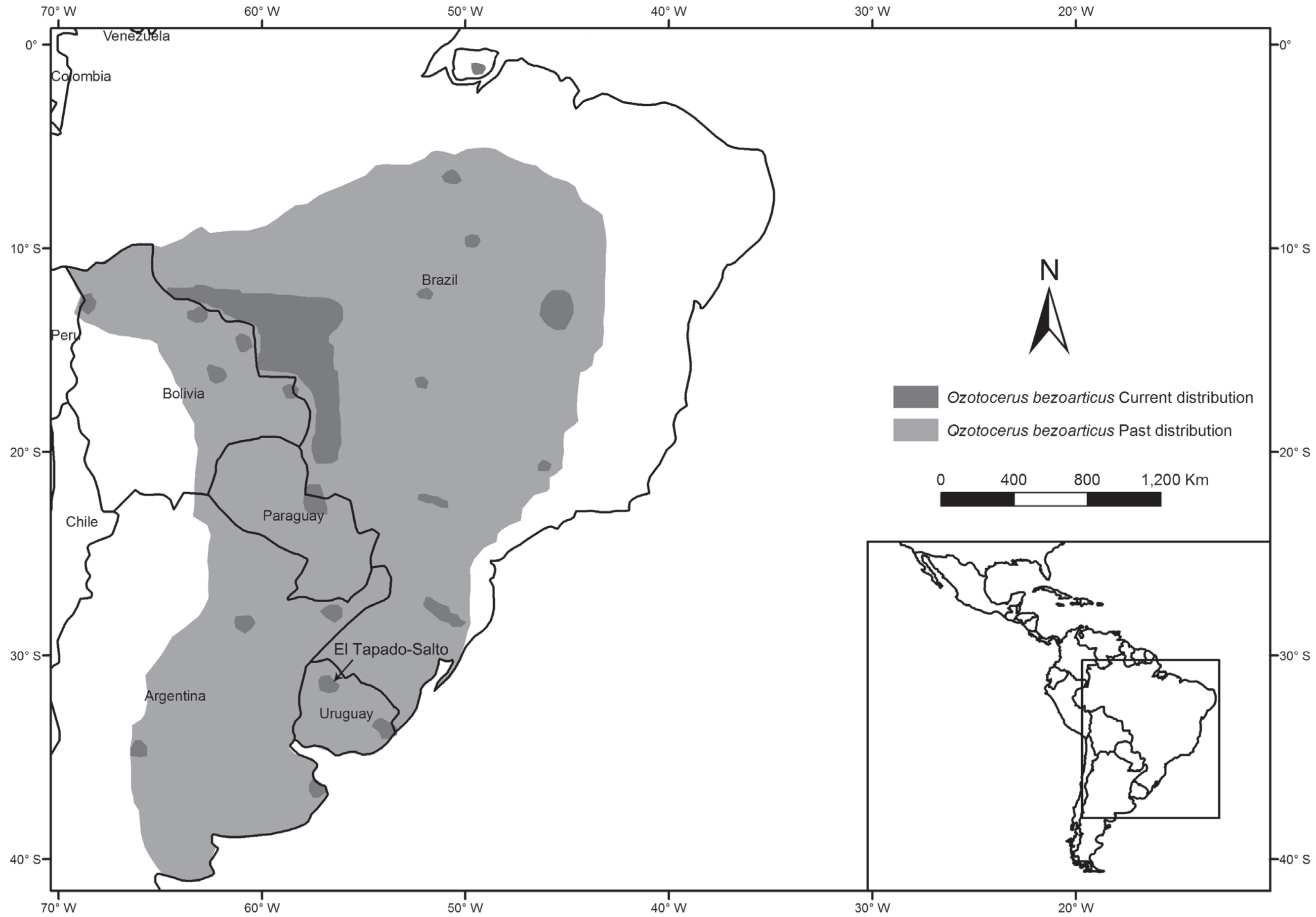

Figure 1. Pampas deer past geographic range and current main populations, showing "El Tapado" ranch geographic location in Uruguay (arrow).

sympatry. There is a previous survey performed by Prestwood *et al*. (1976) on white tailed deer that shared the pastures with sheep. The authors identified 11 parasite species being only five species (45.4%) in both hosts deer and sheep. Those findings strongly suggested that the parasite fauna of deers and sheep are quite distinct, sharing half of the parasite species.

The aim of this study is to describe the gastrointestinal parasites of Pampas deer (*O. b. arerunguaensis*) that share paddock areas with bovine and ovine populations in each season along one year. Using coproparasitological techniques we performed i) morphological analysis of eggs and larvae and, ii) quantification of eggs per gram of feces.

MATERIALS AND METHODS

Study area and animal population sampling

The surveys were conducted seasonally in "El Tapado" ranch, located in Salto (31° 38' 51'' S; 56° 43' 56'' W, Fig. 1) in the Conservation Priority Area "Arerunguá" also devoted to wild fauna and flora conservation, in which a mixed production system takes place where *Corriedale* ovines and *Hereford* bovines are raised together inhabiting with the Pampas deer. The climate is temperate and exhibits marked seasonality, with an average annual temperature of 18.5°C and 1400 mm annual rainfall.

Three paddocks (A, B, C) were selected for conducting the study in August (winter), September (spring) 2012, December (summer) and May (autumn) 2013. The hectares, host species and annual average number of animals (NĀ) by paddock are shown below:

Paddock A – (591 hectares), bovines (*Bos taurus*) over two years old (steers) (NĀ: 345), and Pampas deers (NĀ: 79).

Paddock B – (220 hectares), bovines (*Bos taurus*) less than two years old (calves) (NĀ: 161), and Pampas deers (NĀ: 7).

Paddock C – (316 hectares), ovines (*Ovis aries*) age categories (sheep and lambs) (NĀ: 733), and Pampas deers (NĀ: 10).

A total of 348 fecal samples were analyzed: 119 from adult Pampas deers, 144 from bovines (steers and calves) and 85 from ovines. In Table 1 the total number of animals, the number of sampled feces and the annual average number of animals are shown, discriminated by host population and season in each paddock.

The ovines were oral dosed before the beginning of the survey in May 2012 (autumn) with moxidectin, and on other three occasions during the study period in October 2012 (mixture of ivermectin, levamisole and albendazole), in February 2013 (trichlorphon) and again in April 2013 (moxidectin). Meanwhile, the calves were injected with ivermectin, both before the study started, in July 2012, and during the survey, in March 2013. Steers were injected before the beginning of the survey, in April and July 2012, with nitroxynil and ivermectin whereas, during the survey, did not receive any anthelmintic drug dosage.

During the study, as a wildlife species, Pampas deer did not receive any anthelmintic dosage. We performed transects through the paddocks using the same methodology, collecting the fresh fecal samples at the defecation moment. We surveyed each paddock using binoculars (TASCO® 10 x 50mm), one time per season, collecting fresh fecal samples (approximately 50 g for bovines, and 25 g for ovines and Pampas deers). Each sample was identified- species, date, and paddock name- stored in polyethylene bags, refrigerated (5°C), and sent to coprological analysis at the *Laboratorio de Parasitología Veterinaria* of the *Cenur Litoral Norte Salto*, *Universidad de la República*, Salto, Uruguay.

Laboratory analysis

The coprological studies included a macro and micro examination. A morphological analysis of eggs and larvae was performed. The presence of *Fasciola hepatica* and/or *Paramphistomum* spp. was determined with a qualitative technique of concentration by successive sedimentation following Happich and Boray (1969). Other parasites (e.g., *Nematodirus* spp., *Trichuris* spp., *Moniezia* spp., Coccidia oocysts) were identified by egg morphology (Thienpont et *al*., 1986; Hendrix and Robinson; 2006).

For generic identification of gastrointestinal nematodes having indistinguishable eggs, pools of fecal samples per host species, paddock and season were coprocultured following Roberts and O'Sullivan (1949) and Niec (1968) techniques. The morphology of up to 100 cultured infective larvae of each pooling was analyzed.

The quantification of nematode load was estimated by eggs per gram of feces (epg) in each sample through the Mc Master technique with a sensitivity of 50 epg for ovines and Pampas deers, and 20 epg for bovines (Thienpont *et al*., 1986).

Statistical analysis

The epg descriptive statistic (mean, standard deviation, variance, confidence interval, standard error, and the variation ranges), per ruminant species, age for the case of bovines (calves and steers), was calculated in each paddock and season. Multiple comparisons among seasons, spatial distribution (paddocks) and the epg counting for species were performed, with the Levene's Test for Homogeneity of Variances and ANOVA with two and three-level factors at a confidence level of 95% (Sokal and Rohlf, 1969), using StatSoft (Hill and Lewicki, 2007).

In order to make comparisons among paddocks´ livestock densities, the livestock unit (LU) measurement

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was used, which is a reference index that measures animal stock, facilitating the aggregation of livestock from various species and ages per hectare (Eurostat, 2020). In Uruguay, one LU represents the maintenance requirements of a 380 kg of live weight cow (Crempien, 1982). The density averages during the study period for domestic ruminant hosts were in paddock A: 0.58 LU, in B: 0.72 LU and in C: 0.50 LU. Generally, the highest number of animals per unit area occurred during spring and summer, except for the paddock A where highest stocks were recorded in spring and winter (Table 1).

An estimation of richness (S) of genera of parasites in each host species and category (Pampas deers, steers, calves, and ovines) in each studied paddock was performed following Bush *et al.* (1997). This index is a quantitative measure that reflects the number of parasite taxa in a ruminant host.

RESULTS

Table 2 summarizes the results of parasite genera identified in each paddock, host species and season of year. The proportion and distribution of nematode genera identified by larvae culture are shown in Figure 2.

In paddock A, four parasite genera were recorded in winter, three genera in spring, two genera in summer and only one genus in autumn. There were no genera shared between steers and Pampas deers in the same season. In paddock B, six parasite genera were recorded in winter, three genera in spring, one genus in summer and four genera in autumn. The genus *Oesophagostomum* was shared between calves and Pampas deers in winter. In paddock C, six parasite genera were recorded in winter, six genera in spring, two genera in summer and one genus in autumn. The genera *Haemonchus*, *Oesophagostomum*, *Ostertagia* and *Trichostrongylus* were shared between ovines and Pampas deers in winter and *Haemonchus* in autumn (Table 2).

The greatest parasite generic richness was found in winter parasitizing calves and ovines, being the largest record also observed in this season in the Pampas deers of the paddock C. Notably, in spring, the same parasite taxa were recorded in ovines but Pampas deers did not present parasites. *Fasciola hepatica* was recorded only in calves in summer. Additionally, Coccidia oocysts were observed only in steers, except in summer (these records were not listed in the table). *Paramphistomum* spp. eggs were not found in any of the ruminant species during the surveyed year.

On the other hand, we found moderate to low epg counts per host species and/or animal category, paddock, and season of the year (Table 2). The general mean values obtained were: 348.6 epg in ovines, 74 epg in calves, 3.7 epg in steers, and 38.8 epg in Pampas deers. The statistical comparisons showed that ovines exhibited the highest significant epg counting during spring (796 epg; $p<0.001$). Meanwhile, in calves the highest significant epg counting was detected during winter (203 epg; $p<0.001$), and for the Pampas deer was detected in summer (116 epg). Steers showed the minimum epg values in the four seasons, with no significant differences ($p=0.39$). The epg counting in Pampas deer from the three paddocks showed a similar seasonal behavior throughout the year ($p=0.89$). Only in summer were detected significant differences ($p=0.023$) among paddocks.

In summer, the combination of the anthelmintic dosage and the weather conditions seemed to reduce the parasite taxa in ovines, as well as the epg counting.

Table 1. Total number of animals (Na), sampled feces (Nf) discriminated by host population and season, and annual average number of animals of each species (NĀ) in each paddock and season. Last column indicates the total number of feces analysed for each ruminant population and paddock.

Paddock	Species*	Winter		Spring		Summer		Autumn		NĀ	Total*			
		Na	Nf	Na	Nf	Na	Nf	Na	Nf		Pd	Bov	Ov	Paddock
A	Pampas deers	78	23	53	20	100	22	83	18	79	**83**			**163**
	Steers	350	22	436	20	313	20	281	18	345		**80**		
B	Pampas deers	8	5	6	4	6	3	6	3	7	**15**			**79**
	Calves	107	16	1147	15	251	18	137	15	161		**64**		
C	Pampas deers	8	6	11	5	10	5	11	5	10	**21**			**106**
	Ovines	747	20	744	26	790	19	648	20	733			**85**	
Total											**119**	**144**	**85**	**348**

* Pampas deer (Pd), bovine -including steers and calves (Bov), and ovine (Ov)

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Table 2. Richness of parasite genera (S index) and Mean and SD of eggs per gram (epg) of gastrointestinal nematodes genera by paddock, species hosts and seasons.

Paddock	Species*		Winter	Spring	Summer	Autumn
A	Pampas deers	S index	2	1	2	1
		Genera	*Trichostrongylus* *Oesophagostomum*	*Haemonchus*	*Haemonchus* *Ostertagia*	*Haemonchus*
		epg	15.2	15	93	41
		SD	41.103	32.84	121.78	79.05
	Steers	S index	2	2	0	0
		Genera	*Ostertagia* *Moniezia*	*Trichostrongylus* *Cooperia*		
		epg	10.9	4	0	0
		SD	42.64	13.91	0	0
B	Pampas deers	S index	1	0	1	0
		Genera	*Oesophagostomum*		*Haemonchus*	
		epg	20	0	116	0
		SD	44.72	0	202.07	0
	Calves	S index	6	3	1	4
		Genera	*Cooperia* *Haemonchus* *Oesophagostomum* *Trichostrongylus* *Moniezia* *Trichuris*	Cooperia *Haemonchus* *Oesophagostomum*	*F. hepatica*	*Haemonchus* *Oesophagostomum* *Ostertagia* *Trichostrongylus*
		epg	203	76	13.3	4
		SD	197.03	107.22	29.10	11.21
C	Pampas deers	S index	4	0*	0*	2
		Genera	*Haemonchus* *Oesophagostomum* *Ostertagia* *Trichostrongylus*	*unidentified	*unidentified	*Haemonchus* *Ostertagia*
		epg	16.6	10	100	40
		SD	25.81	22.36	127.47	54.778
	Ovines	S index	6	6	2	1
		Genera	*Haemonchus* *Oesophagostomum* *Ostertagia* *Trichostrongylus* *Moniezia* *Nematodirus*	*Haemonchus* *Oesophagostomum* *Ostertagia* *Trichostrongylus* *Moniezia* *Nematodirus*	*Oesophagostomum* *Ostertagia*	*Haemonchus*
		epg	320	796	181	97.5
		SD	705.69	729.23	229.86	152.58

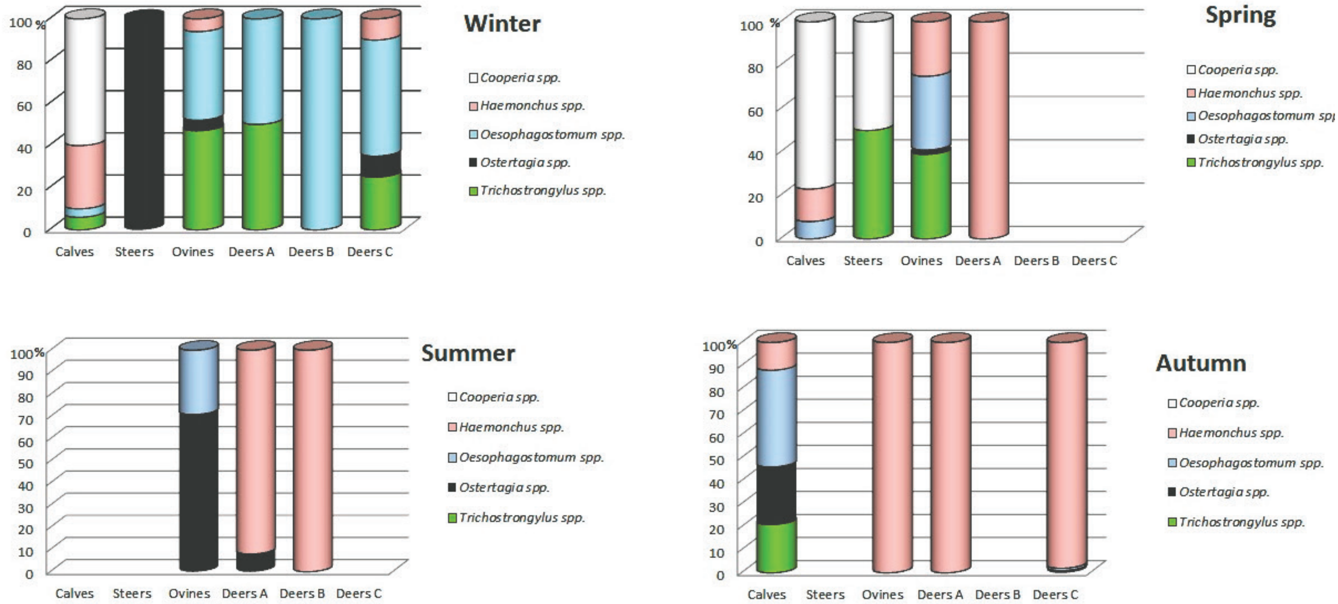

Figure 2. Proportion of the distribution of nematode genera diagnosed with larvae culture in Pampas deers, steers, calves, and ovines for each sampling season, and in each paddock (A, B and C).

On the other hand, in paddock C, parasitic taxa were not detected in the Pampas deer neither in spring nor in summer. Noteworthy, in this paddock, in winter, the Pampas deer showed the highest S index value (S=4) but the epg average counting was low (16.6; SD 25.81).

DISCUSSION

These are the first parasitic data obtained from the endangered Pampas deer as well as domestic ovines and bovines living simultaneously in sympatry. Preliminary studies on the Pampas deers, conducted in another ranch from the Arerunguá area, which did not include domestic ruminants, recorded ten parasite genera, and 74% of the analyzed samples showed loads less than 100 epg (Hernández *et al*., 1994; Hernández and González, 2012). However, in the current study, genera previously detected like *Capillaria*, *Fasciola*, *Moniezia*, *Paramphistomum*, *Strongyloides* and *Trichuris* were not found in Pampas deers. These contrasting results could be related to differences in the management practices applied in both ranches. These practices included the domestic ruminant's anthelmintic dosage schedule and the maintenance of a LU (0.75) lower than the average commonly found in Uruguay.

Similar low parasite values were registered in several deer species, such as axis deer, caribou, red deer, Neotropical deer species from Brazil and white tailed deer (Pursglove *et al*., 1976; Mc Kenzie and Davidson, 1989; Richardson and Demarais, 1992; Nascimento *et al*., 2000; Lux Hoppe *et al*., 2010; Tapia-Escárate *et al*., 2015; Ten Doesschate *et al*., 2017; Turgeona *et al*., 2018).

Moreover, previous research in other wildlife deer species showed the same gastrointestinal parasite genera reported here in domestic hosts (*Haemonchus, Ostertagia, Trichostrongylus, Trichuris, Nematodirus, Cooperia,* and *Oesophagostomum*; Prestwood *et al*., 1975; Cook *et al*., 1979; Romero-Castañón *et al*., 2008; Pato *et al*., 2009, 2013).

In red deer the establishment rate of parasite infections was generally low in comparative with sheep and cattle, not being a successful host for *Cooperia oncophora* and *Ostertagia ostertagi*; but it may be easily infected with *Haemonchus contortus* and *Trichostrongylus axei* (Tapia-Escárate *et al*., 2015; Ten Doesschate *et al*., 2017). These studies highlighted the red deer susceptibility to Haemonchinae, as we also detected a clear predominance of *Haemonchus* spp. in Pampas deers in summer, coinciding with an increase of the epg counts. In this regard, Campbell and VerCauteren (2011) considered that most cervids act as hosts of *H. contortus* without showing signs of disease, and those who experience haemonchosis are young animals that are often infected with other parasites as well. Also, under experimental conditions, *H. contortus* is able to be transferred between white-tailed deer and domestic livestock (McGhee *et al*., 1981). Molecular genetic analysis has supported this finding by suggesting that sheeps, wild bovids and wild deers share a common field population of *H. contortus* (Cerutti *et al*., 2010).

The parasite dynamic described in this study was quite similar to those reported by Prestwood *et al*. (1976) in white tailed deer in sympatry with sheep in the USA, that do not share at the same season the same endoparasite taxa. Specifically, we have not detected any parasite taxa from Pampas deer sharing paddock C with ovines, neither in spring nor in summer. However, it is important to highlight that ovines had received anthelmintic dosage treatment and this, added to the summer weather conditions, could have contributed to reducing the parasite taxa in ovines, as well as the epg counting.

Finally, considering the agroecosystem scenario, parasitic diseases do not seem to be an immediate threat for the Pampas deer survival in Uruguay. However, the populations need to be closely monitored for assuring the mid-term viability. The great pathogenicity and the high reproductive capacity of *Haemonchus* have been reported in livestock, being one of the predominant nematodes in this study. When the climate conditions benefit the parasite in the warm season, the infestation rates can be very high and cause death in livestock in a few weeks (Fiel and Nari, 2013). In the case of the Arerunguá region, it is recommended to reduce stock densities on ranches that have large sheep numbers, being advisable not to overload to 0.6 to 0.8 LU. Furthermore, another recommended measure will be to leave some un-stock paddocks in order to separate Pampas deer from domestic stock. We endorse controlling the parasitic infections mainly in sheep and calves, as well as regulating the animal domestic densities for avoiding the grassland contamination and the cross transmission, tending to assure the Pampas deer viability and welfare conditions.

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