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Fasciola hepatica infection and association with gastrointestinal parasites in Creole goats from western Argentina

Fasciola hepatica infecção e associação com parasitas gastrintestinais em caprinos crioulos do oeste da Argentina

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Abstract

Goats, called "the cow of the poor", are the livestock species with the most significant population growth worldwide in recent years. Gastrointestinal parasitism constitutes one of the main constraints to its outdoor and extensive breeding in temperate and tropical countries. Despite a Creole goat population of nearly 4 million heads, local reports on parasitological prevalence are scarce, and while *Fasciola hepatica* infection is spread all over Argentina, the goat is usually neglected as a reservoir and economic losses are not considered. To evaluate gastrointestinal parasitism prevalence and associations between parasite genera and species, with emphasis on fascioliasis, Creole goats from the plateau and Andean regions from western Argentina were investigated by coprological techniques, and associations were statistically assessed. Eighty-five percent (85%) of the animals harbored one or more parasite types, while 46% showed mixed infections. Significant positive associations between *F. hepatica* + Strongyle eggs, *Eimeria* sp. + *Nematodirus* sp. and *Nematodirus* sp. + *Trichuris ovis* were detected. Further studies are required to define the causality of these associations and their relevance in epidemiology. *F. hepatica* is rarely considered as goat parasite in the country, but a 33% prevalence poses an interrogation on the role goats play on the transmission and dissemination of this zoonotic trematode.

Keywords: Caprine, fascioliasis, Wertern Argentina, *Eimeria* sp., *Nematodirus*, *Trichuris ovis*.

Resumo

As cabras, nomeadas como "a vaca dos pobres", são as espécies de gado com o crescimento populacional mais significativo nos últimos anos em todo o mundo. O parasitismo gastrintestinal constitui uma das principais limitações à sua criação extensiva em clima temperado e tropical. Na Argentina, apesar de uma população de caprinos crioulos de cerca de quatro milhões de cabeças, são escassos os relatórios locais de prevalências parasitológicas. Embora a infecção por *Fasciola hepatica* esteja espalhada em todo o país, as cabras são geralmente negligenciadas como um reservatório, e as perdas econômicas não são consideradas. Para avaliar a prevalência do parasitismo gastrintestinal e associações entre os gêneros e espécies de parasitos, com ênfase na fasciolose, caprinos crioulos da região andina e do planalto do oeste de Argentina foram avaliados por meio de técnicas coprológicas. Oitenta e cinco por cento dos animais hospedaram um ou mais tipos de parasitos, enquanto 46% hospedaram infecções mistas. Foram encontradas associações significativas entre *F. hepatica* + ovos de estrongilídeos, *Eimeria* sp. + *Nematodirus* sp. e *Nematodirus* sp. + *Trichuris ovis*. Mais estudos são necessários para definir a causalidade dessas associações e sua relevância na epidemiologia. Raramente *F. hepatica* é considerada como um parasito de cabra no país, mas uma prevalência de 33% suscita uma interrogação sobre o papel dos caprinos na transmissão e disseminação desse trematódeo zoonótico.

Palavras-chave: Caprinos, fasciolose, Oeste da Argentina, *Eimeria* sp., *Nematodirus*, *Trichuris ovis*.

Introduction

Goat exploitation is an example of a sustainable production that is fully integrated within the local rural development, being the domestic livestock species with the most significant population growth worldwide in recent years, mainly in developing countries (MORAND-FEHR et al., 2004; BOYAZOGLU et al., 2005). Its current success appears to be related with two characteristics: i) goats are efficient converters of low-quality forages into quality products, and ii) constitute a source of high quality protein; both of them high valuable properties in farming systems with limited resources (LEBBIE, 2004; BOYAZOGLU et al., 2005), such as those present in most developing countries (LEBBIE, 2004). These characteristics may explain the frequent labeling of goats as "the

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cow of the poor", highlighting its importance in small farming systems (BOYAZOGLU et al., 2005; HOSTE et al., 2010).

Gastrointestinal parasitism constitutes one of the main constraints to the outdoor and extensive breeding of goats in temperate and tropical countries (HOSTE et al., 2005, 2008; COELHO et al., 2012). For years, it has been considered that data obtained on parasite infections in sheep could be directly transferred to goats (HOSTE et al., 2010), and thus disregarding specific studies on goat parasitism and its consequences. However, in recent years, accumulated information have underlined the fact that gastrointestinal parasitism in goats differed in many aspects to that in sheep (HOSTE et al., 2008), and thus studies in the area are encouraged (SAHLU; GOETSCH, 2005).

The goat population in Argentina reaches about 4 million heads, mainly distributed in the harsh and dry environments of the central and western regions. The dominant flocks are of Creole goats, derived from introductions during the Spanish colonial times, and mainly owned by small holders, usually belonging to the most marginalized sector of the population. Animals are raised extensively, grazing rangelands during the day and housed during the nights in rustic corrals near the households (DE GEA et al., 2005).

Fascioliasis, caused in the Americas by the trematode *Fasciola hepatica* (MAS-COMA et al., 2009), produces a serious economic impact (reduction in milk and meat production, liver condemnation, reproductive failure and mortality) in the livestock industry (KAPLAN, 2001; SCHWEIZER et al., 2005). Despite the mentioned economic impact and an emergent, potentially important, goat production in South America (DUBEUF et al., 2004), goat fascioliasis is mainly neglected in the sub-continent. In Argentina, according to official slaughterhouse records, animal fascioliasis covers the whole country (MERA Y SIERRA et al., 2011), but this information only comprises cattle and sheep, and rarely involves goats. Despite goat fascioliasis in the country not being even considered (ROSSANIGO, 2007), there are few, usually opportunistic, reports of infection, but demonstrating high local prevalence rates (AGUIRRE et al., 2005; RUBEL et al., 2005; ISSIA et al., 2009).

The aim of this study was to determine i) the prevalence rates of gastrointestinal parasites, ii) single and multiple infections, and iii) statistical associations, with special emphasis on liver fluke *F. hepatica*, in Creole goats from the plateau and Andean regions of western Argentina

Materials and Methods

1. *Feces collection and laboratory methods*

Six hundred sixty-three (663) Creole goats born in the plateau and Andean regions of Mendoza (623), San Juan (16) and La Rioja (24) provinces (western Argentina) were surveyed between June 2006 and December 2011. Samples were sent by private practitioners, who requested coproparasitological analysis. Individuals were selected by convenience and fecal samples were directly collected from the rectum. The collected samples were labeled, refrigerated and transported to the laboratory for examination. Coproparasitological exams were systematically performed by means of three methods: Sheater's flotation technique (SHEATHER, 1923), Ritchie's formol-ether concentration technique (RITCHIE, 1948) and Lumbreras technique (LUMBRERAS et al., 1962). Fecal culture and larvae identification were performed when sufficient material from positive samples was available (NIEC, 1968).

2. *Statistical analysis*

Statistix® for Windows 7.0 and SPSS® for Windows 17.0 software programs were used for statistical analysis, comparison of categorical variables and chi-square test. Chi-square test was applied for comparison between observed and expected prevalence.

Associations between *F. hepatica* and other parasite types were investigated by 2 × 2 contingency tables, from which the chi-square was calculated. First, each parasite type occurrence was compared to each other; and then, *F. hepatica* occurrence was compared with pairs of parasite types (in each threesome, all combinations possible were tested). Due to small N and loss of statistical power, no further comparisons were investigated. Values of $P < 0.05$ were taken as significant. Odds Ratio (OR), OR confidence interval (95%) and Relative Risk (RR) were calculated in cases where significant positive association was detected. Considering that, unlike OR, the calculation of RR is influenced by the position of the variable in the 2 × 2 contingency table, both combinations were tested.

Results

Out of the total 663 goats examined, 84.8% (562) were found to host one or more parasite types (as described in Table 1). Among the infected animals, 46% hosted mixed infections. 64.7% of the animals were infected with a protozoan species and 63.49% with at least one helminth species. Two hundred and seventeen (32.9%) of the examined animals (659) were positive for *F. hepatica*, 344 (51.88%) were positive for nematodes, while 422 (64.72%) were positive for *Eimeria* sp. oocysts (Table 1).

Following the few reports available (AGUIRRE et al., 2005; RUBEL et al., 2005; ISSIA et al., 2009), expected prevalence could only be determined for *F. hepatica* infection (94.7%), being the observed prevalence (32.9%) significantly lower than expected ($P<0.05$).

The prevalence rates of single and multiple parasite type infections are shown in Table 2. Considering presence of parasite types, single presence (38.61%) was the most common, with *Eimeria* sp. as the predominant type (21.27%), followed by double presence (31.82%). The most frequent combinations were *Eimeria* sp. + *Nematodirus* sp. (15.38%) and *Fasciola hepatica* + *Eimeria* sp. (11.01%).

F. hepatica occurred mainly as a co-infection with another parasite, while only 22.12% (48) of the total cases of fascioliasis (217) occurred as monoparasitism (Table 2). It was most frequently combined with *Eimeria* sp. (11.01%), followed by the duet *F. hepatica* + *Nematodirus* sp. (2.11%), and lastly combined with Strongyle eggs (0.90%).

Table 1. Observed prevalence rates of parasite infection among 663 Creole goats in the plateau and Andean regions, Argentina (overall and per province surveyed).

Parasites	*N*	% Infect. (CI 95)	Mza (%)	S. Juan (%)	La Rioja (%)
All	663	84.8 (82.2-87.3)	623	16	24
Strongyle eggs	652	9 (6.9-11)	42 (6.74)	0	17 (70.83)
Nematodirus sp.	663	40.6 (37.1-44.1)	265 (42.54)	2 (12.5)	2 (8.33)
T. ovis	663	2.3 (1.2-3.4)	15 (5.7)	0	0
F. hepatica	659	32.9 (29.6-36.2)	208 (33.39)	0	9 (37.5)
Eimeria sp.	652	64.7 (61.3-68.1)	414 (66.45)	1 (6.25)	7 (29.17)

% Infect.: % of infection; Mza: Mendoza province; S. Juan: San Juan province.

Table 2. Combination of infecting parasites among 663 Creole goats in the plateau and Andean regions, Argentina (combinations with less than 0.5% are not shown).

Parasites of combination	Nº (%)
Any single type	256 (38.61)
Strongyle eggs	8 (1.21)
Nematodirus sp.	59 (8.90)
F. hepatica	48 (7.24)
Eimeria sp.	141 (21.27)
Double combination	211 (31.82)
F. hepatica + *Eimeria* sp.	73 (11.01)
F. hepatica + Strongyle eggs	6 (0.90)
F. hepatica + *Nematodirus* sp.	14 (2.11)
Eimeria sp. + Strongyle eggs	11 (1.66)
Eimeria sp. + *Nematodirus* sp.	102 (15.38)
Triple combination	77 (11.61)
F. hepatica + *Eimeria* sp. + *Nematodirus* sp.	53 (7.99)
Eimeria sp. + Strongyle eggs + *Nematodirus* sp.	10 (1.51)
Eimeria sp. + *Nematodirus* sp. + *T. ovis*	8 (1.21)
Quadruple combination	17 (2.56)
F. hepatica + *Eimeria* sp. + Strongyle eggs + *Nematodirus* sp.	16 (2.41)

Due to insufficient material (feces) and low eggs per gram (mean 158.4 epg), results from fecal culture for larvae identification were obtained only with the positive samples from La Rioja province. *Trichostrongylus* sp. were identified in every sample cultured (15), while 9 samples were positive to *Haemonchus* sp., 2 to *Oesophagostomum* sp. and 1 to *Ostertagia* sp. The overall prevalence rates found was: *Trichostrongylus* sp. 95.81%, *Haemonchus* sp. 3.11%, *Oesophagostomum* sp. 0.95% and *Ostertagia* sp. 0.14%.

Chi-square association tests revealed significant positive associations between *F. hepatica* and Strongyle eggs, *Eimeria* sp. and *Nematodirus* sp., and *Nematodirus* sp. and *T. ovis* (Table 3).

When *F. hepatica* occurrence was compared with pairs of parasite types and possible combinations, significant positive associations were found in the following threesomes: *F. hepatica* + *Eimeria* sp. + *Nematodirus* sp., *F. hepatica* + *Eimeria* sp. + Strongyle eggs, and *F. hepatica* + Strongyle eggs + *Nematodirus* sp. (See Table 3 for details).

Discussion

To the authors' knowledge, this study is the first to report precise goat parasites prevalence rates in western Argentina. As usual, and in accordance with previous world tendencies, goat parasitism in the country has been frequently disregarded, and such valuable information not informed due to general disinterest. Most of the available local studies only informed about seasonality of gastrointestinal parasites fecal counts and treatment results, but gave no information about local prevalence that allowed further epidemiological comparison.

This study reveals a high overall prevalence of gastrointestinal parasites, reaching almost 85% - maximum for *Eimeria* sp. (64.7%) and minimum for *Trichuris ovis* (2.3%). Meanwhile, the high prevalence (32.9%) of *F. hepatica* is remarkable.

Due to the mentioned scarcity of local data regarding prevalence of goat gastrointestinal parasitism, observed and expected values could only be compared in the case of infection by *F. hepatica*, which was significantly lower than expected ($P < 0.05$) (Table 1). In the few national reports where *F. hepatica* infection in goats was described (AGUIRRE et al., 2005; RUBEL et al., 2005; ISSIA et al., 2009), studies were developed in response to outbreaks, sample numbers were small, and individuals sampled belonged to the same herd, thus explaining the very high local prevalence and the consequent difference between observed and expected values.

The status of polyparasitism using coproscopy as the method indicated that almost half (46.1%) of these animals harbored 2-5 different parasite eggs. Despite the numerous parasite combinations observed (Table 2), significant positive associations ($P < 0.05$) were detected only between *F. hepatica* + Strongyle eggs, *Eimeria* sp. + *Nematodirus* sp. and *Nematodirus* sp. + *T. ovis* (Table 3).

In the first case, *F. hepatica* + Strongyle eggs, the association could be non-causal (confounding factor), possibly due to favorable environmental characteristics for the development of larvae and infection in the same places where infection with *F. hepatica* occurs (e.g. swamps and waterlogged areas). On the other hand, the OR and RR analysis suggests that infection by *F. hepatica* may act as a contributing factor for Strongyle infection, enhancing almost twice (1.96 and 1.83 respectively) the odds, probably due to the host immunosuppression attributed to the trematode (CERVI et al., 1996; SIDOTI, 2011) or just due to a more vulnerable host (a host's weakened state) (BEGON et al, 2006). Considering that most of the larvae identified belonged to the family Trichostrongylidae, association could also be attributed to the arrested larvae released by the suggested immunosuppression (ANDERSON, 2000).

Table 3. Associations between gastrointestinal parasite among 663 Creole goats in the plateau and Andean regions, Argentina.

Parasite sp.	χ^2	*P*-value	OR (CI 95%)
*F. hepatica** Strongyle eggs	6.11	0.0134	1.96 (1.14-3.36)
Eimeria sp.* *Nematodirus* sp.	7.91	0.0049	1.61 (1.15-2.25)
Nematodirus sp.* *T. ovis*	9.89	0.0017	6.09 (1.70-21.78)
Eimeria sp.* *F. hepatica* + *Nematodirus* sp.	12.54	0.0004	2.70 (1.53-4.78)
Nematodirus sp.* *F. hepatica* + *Eimeria* sp.	4.68	0.0306	1.50 (1.04-2.17)
*F. hepatica** *Eimeria* sp. + Strongyle eggs	4.38	0.0363	1.94 (1.03-3.63)
Strongyle eggs* *F. hepatica* + *Eimeria* sp.	4.79	0.0287	1.88 (1.06-3.34)
*F. hepatica** Strongyle eggs + *Nematodirus* sp.	13.2	0.0003	3.76 (1.76-8.05)
Strongyle eggs* *F. hepatica* + *Nematodirus* sp.	19.9	0.0000	3.67 (2.01-6.69)
Nematodirus sp.* *F. hepatica* + Strongyle eggs	9.03	0.0027	3.25 (1.45-7.30)

* Significant associations.

The significant association observed between *Eimeria* sp. + *Nematodirus* sp. and *Nematodirus* sp. + *T. ovis* could be a consequence of infective stages (oocysts and eggs) resistant to extreme environmental conditions or related to housing conditions, with animals returning to corrals every day and manure being accumulated during months.

When complex associations were evaluated, significant positive associations were detected in three sets of combinations: *F. hepatica* + *Eimeria* sp. + *Nematodirus* sp., *F. hepatica* + *Eimeria* sp. + Strongyle eggs, and *F. hepatica* + Strongyle eggs + *Nematodirus* sp. (Table 3). In the first two threesomes, the association observed is attributed to the significant associations detected when analyzing by pairs (*Eimeria* sp. + *Nematodirus* sp. and *F. hepatica* + Strongyle eggs), even more when the third parasite type (*F. hepatica* and *Eimeria* sp., respectively) is tested against the associated pair and no significant association is detected. It is worth noting the last threesome, where, following the previous criteria, the presence of eggs of *Nematodirus* sp. is significantly associated with the combination *F. hepatica* + Strongyle eggs ($\chi^2 = 9.03$, $P = 0.0027$).

Further studies are required to define whether these associations are causal or not, and their relevance in the epidemiology of the parasite types implicated. Future studies should also analyze the economic losses generated by goat gastrointestinal multiparasitism, especially considering its impact on local extensive small farming systems.

As highlighted before, *F. hepatica* is rarely considered as a parasite of goats in the country. Consequently, not only the recognized economic losses are not analyzed, but also this ruminant is neglected as an important reservoir of this zoonosis. Furthermore, the stunning 33% prevalence detected poses an interrogation and alerts about the role goats play on the transmission and dissemination of this zoonotic trematode.

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References

Aguirre DH, Cafrune MM, Salatin AO, Abeyá AA. Fasciolosis clínica en cabras de Metán (Salta, Argentina). *Rev FAVE – Cienc Vet* 2005; 4(1-2): 17-21.

Anderson RC. *Nematode parasites of vertebrates:* Their development and transmission. 2nd ed. Wallingford: CAB International; 2000. http://dx.doi.org/10.1079/9780851994215.0000

Begon M, Townsend CR, Harper JL. *Ecology*: from individuals to ecosystems. 4th ed. Oxford: Blackwell Publishing; 2006.

Boyazoglu J, Hatziminaoglou I, Morand-Fehr P. The role of the goat in society: past, present and perspectives for the future. *Small Ruminant Res* 2005; 60(1-2): 13-23. http://dx.doi.org/10.1016/j.smallrumres.2005.06.003

Cervi L, Rubinstein H, Masih DT. Involvement of excretion-secretion products from *Fasciola hepatica* inducing suppression of the cellular immune responses. *Vet Parasitol* 1996; 61(1-2): 97-111. http://dx.doi.org/10.1016/0304-4017(95)00816-0

Coelho WM, Amarante AF, Bresciani KD. Ocurrence of gastrointestinal parasites in goat kids. *Rev Bras Parasitol Vet* 2012; 21(1): 65-67. PMid:22534948. http://dx.doi.org/10.1590/S1984-29612012000100013

De Gea G, Petryna AM, Mellano A, Bonvillani A, Turiello P. *El ganado caprino en la Argentina:* Antecedentes para su estudio. Córdoba: Universidad Nacional de Río Cuarto; 2005.

Dubeuf JP, Morand-Fehr P, Rubino R. Situation, changes, and future of goat industry around the world. *Small Ruminant Res* 2004; 51(2): 165-173. http://dx.doi.org/10.1016/j.smallrumres.2003.08.007

Hoste H, Torres-Acosta JF, Paolini V, Aguilar-Caballero A, Etter E, Lefrileux Y, et al. Interactions between nutrition and gastrointestinal infections with parasitic nematodes in goats. *Small Ruminant Res* 2005; 60(1-2): 141-151. http://dx.doi.org/10.1016/j.smallrumres.2005.06.008

Hoste H, Torres-Acosta JF, Aguilar-Caballero AJ. Nutrition-parasite interactions in goats: is immunoregulation involved in the control of gastrointestinal nematodes? *Parasite Immunol* 2008; 30(2): 79-88. PMid:18186768.

Hoste H, Sotiraki S, Yan Landau S, Jackson F, Beveridge I. Goat-nematode interactions: think differently. *Trends Parasitol* 2010; 26(8): 376-381. PMid:20488752. http://dx.doi.org/10.1016/j.pt.2010.04.007

Issia L, Pietrokovsky S, Sousa-Figueiredo J, Russell Stothard J, Wisnivesky-Colli C. *Fasciola hepatica* infection in livestock flocks, guanacos and coypus in two wildlife reserves in Argentina. *Vet Parasitol* 2009; 165(3-4): 341-344. PMid:19700244. http://dx.doi.org/10.1016/j.vetpar.2009.07.011

Kaplan RM. *Fasciola hepatica*: a review of the economic impact in cattle and considerations for control. *Vet Ther* 2001; 2(1): 40-50. PMid:19753697.

Lebbie SHB. Goats under household conditions. *Small Ruminant Res* 2004; 51(2): 131-136. http://dx.doi.org/10.1016/j.smallrumres.2003.08.015

Lumbreras H, Cantella R, Burga R. Acerca de un procedimiento de sedimentación rápida para investigar huevos de *Fasciola hepatica* en las heces, su evaluación y uso en el campo. *Rev Med Peru* 1962; 31(332): 167-174.

Mas-Coma S, Valero MA, Bargues MD. Chapter 2. *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. *Adv Parasitol* 2009; 69: 41-146. http://dx.doi.org/10.1016/S0065-308X(09)69002-3

Mera y Sierra R, Agramunt VH, Cuervo P, Mas-Coma S. Human fascioliasis in Argentina: retrospective overview, critical analysis and baseline for future research. *Parasite Vectors* 2011; 4: 104. PMid:21663691 PMCid:3141741. http://dx.doi.org/10.1186/1756-3305-4-104

Morand-Fehr P, Boutonnet JP, Devendra C, Dubeuf JP, Haenlein GFW, Holst P, et al. Strategy for goat farming in the 21st century. *Small Ruminant Res* 2004; 51(2): 175-183. http://dx.doi.org/10.1016/j.smallrumres.2003.08.013

Niec R. *Cultivo e identificación de larvas infectantes de nemátodes gastrointestinales del bovino y ovino.* Buenos Aires: INTA; 1968. Manual técnico n. 3.

Ritchie LS. An ether sedimentation technique for routine stool examination. *Bull US Army Med Dep* 1948; 8(4): 326.

Rossanigo CE. Parasitosis de las cabras. In: Suárez VH, Olaechea FV, Rossanigo CE, Romero JR. *Enfermedades parasitarias de los ovinos y otros rumiantes menores en el cono sur de América*. EEA Anguil, INTA; 2007. p. 247-270.

Rubel D, Prepelitchi L, Kleiman F, Carnevale S, Wisnivesky-Colli C. Estudio del foco en un caso de fasciolosis humana en Neuquén. *Medicina* 2005; 65(3): 207-212.

Sahlu T, Goetsch AL. A foresight in goat research. *Small Ruminant Res* 2005; 60(1-2): 7-12. http://dx.doi.org/10.1016/j.smallrumres.2005.06.002

Schweizer G, Braun U, Deplazes P, Torgerson PS. Estimating the financial losses due to bovine fasciolosis in Switzerland. *Vet Rec* 2005; 157(7): 188-193. PMid:16100368.

Sheather AL. The detection of intestinal protozoa and mange parasites by a flotation technique. *J Comp Pathol* 1923; 36: 266-275. http://dx.doi.org/10.1016/S0368-1742(23)80052-2

Sidoti LE. *Diagnóstico de Fascioliasis bovina mediante coprología y serología, y relación con parámetros hematológicos y bioquímicos* [Thesis]. Mendoza: Facultad de Ciencias Veterinarias y Ambientales, Universidad Juan A. Maza; 2011.