

Copro-prevalence of *Fasciola hepatica* in Chilean breed horses in the province of Concepción, Chile

Copro-prevalência de *Fasciola hepatica* em equinos de raça chilena na província de Concepción, Chile

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Abstract

The aim of this research was to assess the copro-prevalence of *Fasciola hepatica* in owned Chilean breed horses (*Equus caballus*) residing in the province of Concepción, Chile. The study was carried out throughout October 2017. Samples were taken from all (100%) Chilean breed horse (124 specimens; 45 females and 79 males, aged between 7 months and 24 years old) from the Concepción province. A rectal stool sample was obtained from each animal. This was analyzed using the Army Medical School method (AMS III) technique to detect *F. hepatica* eggs. Information about the horse's age, sex, previous fasciolicide application, and an assessment of body condition was also obtained. Fisher tests were carried out to analyze the results. Ten horses (8.06%) were positive for the presence of *F. hepatica* eggs. No significant difference of copro-prevalence was found between age (young 8.47%, old 7.69%), sex (female 11.1%, male 6.33%), the previous application of fasciolicide (dewormed 10%, not dewormed 7.89%), or body condition (low condition 11.1%, high condition 0.7%) categories (Fisher tests: $P > 0.05$ in all cases). The results of this study suggest that Chilean breed horses constitute part of the reservoir of *F. hepatica* for both ruminant populations and humans in the Concepción province.

Keywords: *Fasciola hepatica*, prevalence, equine, AMS III, neglected.

Resumo

O objetivo desta pesquisa foi avaliar a copro-prevalência de *Fasciola hepatica* em equinos chilenos (*Equus caballus*), residentes na província de Concepción, região de Bío-Bío, Chile. O estudo foi realizado durante o mês de outubro de 2017. Foram coletadas amostras de todos os equinos estabulados (124 espécimes; 45 fêmeas e 79 machos, com idades entre 7 meses e 24 anos) da província de Concepción. Uma amostra de fezes transretal foi obtida de cada animal, analisada no Laboratório "Dr. Luis Rubilar", da Universidad de Concepción, usando-se a técnica AMS III, para detectar a presença de ovos de *F. hepatica*. O diagnóstico fecal foi complementado com informações sobre a idade do animal, sexo, aplicação prévia de fasciolicida e uma avaliação da condição corporal. Testes de Fisher foram realizados para analisar os resultados. Dos 124 equinos, 10 (8,06%) foram positivos para ovos de *F. hepatica*. Não foi encontrada associação significativa entre idade, sexo, aplicação prévia de fasciolicida ou condição corporal com a presença de ovos. Um cavalo tratado com triclabendazol foi positivo para ovos de *F. hepatica*.

Palavras-chave: *Fasciola hepatica*, prevalência, equinos, AMS III, negligenciada.

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Fasciolosis is a hepatic food-borne trematodosis caused by *Fasciola hepatica* in Chile. It is considered as a neglected tropical disease by the World Health Organization, and it is not only considered a re-emergent parasitosis in some regions (Klaus et al., 2017) causing disease in humans on its own, but it has also reportedly harbored a novel *Neorickettsia* endobacterium closely related to the etiological agents of human Sennetsu and Potomac horse fevers (McNulty et al., 2017). In horses (*Equus caballus*), chronic disease is the most common fasciolosis, primarily resulting in weight loss and a dull hair coat, although a lack of energy, colic, anemia, jaundice and reduced sports performance have been reported (Alcaíno & Apt, 1989). Fasciolosis is uncommon in horses (Raue et al., 2017), as equines are apparently resistant to this trematode. However, some studies have reported this parasite in horses, but most of these are older studies, which has allowed fasciolosis to be characterized as a neglected form of equine parasitosis (Williams & Hodgkinson, 2017). Most related studies conducted around the world have reported low prevalence rates of this parasite (e.g. Quigley et al., 2017), with the exception of one study carried out in Uruguay (Sanchis et al., 2015), which can be explained by the difficult diagnosis (Nelis et al., 2010). In Chile, equine fasciolosis has also been reported with low prevalence rates and in older studies, in such a way that it could be considered a neglected equine parasitosis in this country. Equine *F. hepatica* infection has been reported throughout the entire country, except in the Magallanes y la Antártida Chilena Region and Rapa Nui (Eastern Island). Although the prevalence of equine *F. hepatica* reported in the Biobío Region is low (4.9%), this region has among the highest prevalence rates of the infection in ovine and caprine animals in the country based on slaughter records (Morales et al., 2000). Most prevalence studies of *F. hepatica* have been performed with thoroughbred horses (e.g. Muñoz et al., 2008), and there are no published studies of fasciolosis in Chilean breed horses. Further, there are a couple of theses that are more than 30 years old; however, none of these investigated horses in the Concepción province. Thus, there is scant knowledge of the epidemiology of *F. hepatica* infection in Chilean breed horses and in the Concepción province.

Fourteen farmyards featuring Chilean breed horses can be found in the Concepción province, Biobío Region, where more than 100 horses are harbored. While details related to the distribution and management of those horses (for instance, their sex and age distribution, antiparasitic management, and so forth) have not been published, but it is well-known that many horses share pasturelands with ruminants, enhancing the transmission of *F. hepatica*.

The Concepción province encompasses three different geographical areas: a mountain territory, the 'La Costa mountain range'; the 'coastal plain', a narrow strip of flat lowland between the La Costa range and the Pacific Ocean; and a little part of the 'Valle Central', another lowland at the east of the La Costa range. The Chilean horses' farmyards are located in the lowlands, mostly in the coastal plain, where the presence of *F. hepatica* is expected given that there is a high likelihood of the presence of stagnant water, which likely favors the abundance of the intermediate host, *Galba truncatula* (Artigas et al., 2011). *Galba truncatula* is the primary intermediate host in Europe (Beesley et al., 2018). Thus, the aim of this study was to assess the copro-prevalence of fasciolosis in owned Chilean breed horses from the Concepción province, in the Bío-Bío region, Chile, and to analyze its association with the sex, age, body condition, food source (including pastures), and previous deworming of horses.

The study was performed with the entire population of Chilean breed horses from the Concepción province throughout October 2017. This population comprised 124 horses spanning 14 farmyards in 7 localities: Criaderos Talcahuano (number of horses: 6), Criaderos Hualpén (29), Club de Rodeo Coronel (39), Medialuna Santa Juana (19), Medialuna Hualqui (20), Criadero Penco (4), Medialuna Copiulemu (7) (Figure 1). Among the horses, there were 45 females and 79 males (37 of which were castrated). The female horses were between the ages of 7 months and 24 years old, while male horses were between 7 months and 21 years old.

Horse caretakers and/or owners were surveyed in order to collect information about the following horse characteristics: horse identification; food sources (asking whether the horses fed on pastures as part of their food source); sex; age (years); and whether the horse had been dewormed against *F. hepatica* in the last 60 days.

The horse's body condition was assessed by a single trained veterinary surgeon throughout the study using the 0–5 scale proposed by Carroll and Huntington (Carroll & Huntington, 1988).

A single fecal sample was obtained from the rectum of each horse, kept at 4 °C, and examined over the course of the next 24 hours in the "Dr. Luis Rubilar" Laboratory of Parasitology at the Facultad de Ciencias Veterinarias, Universidad de Concepción. The Army Medical School (AMS III) technique was used to search for *F. hepatica* eggs. In short, the AMS III solution was prepared with a solution of 28% HCl (45 mL) combined with water (55 mL), which was mixed with a solution of Na₂SO₄ (9.6 g) that was previously dissolved in water (100 mL). Then, 1 g of feces was dissolved in 14 mL of tap water, homogenized, filtered, placed into a centrifuge tube, and centrifugated at 448 RCF for 1 minute. The supernatant was discarded and the sediment was mixed and homogenized vigorously with 10 mL

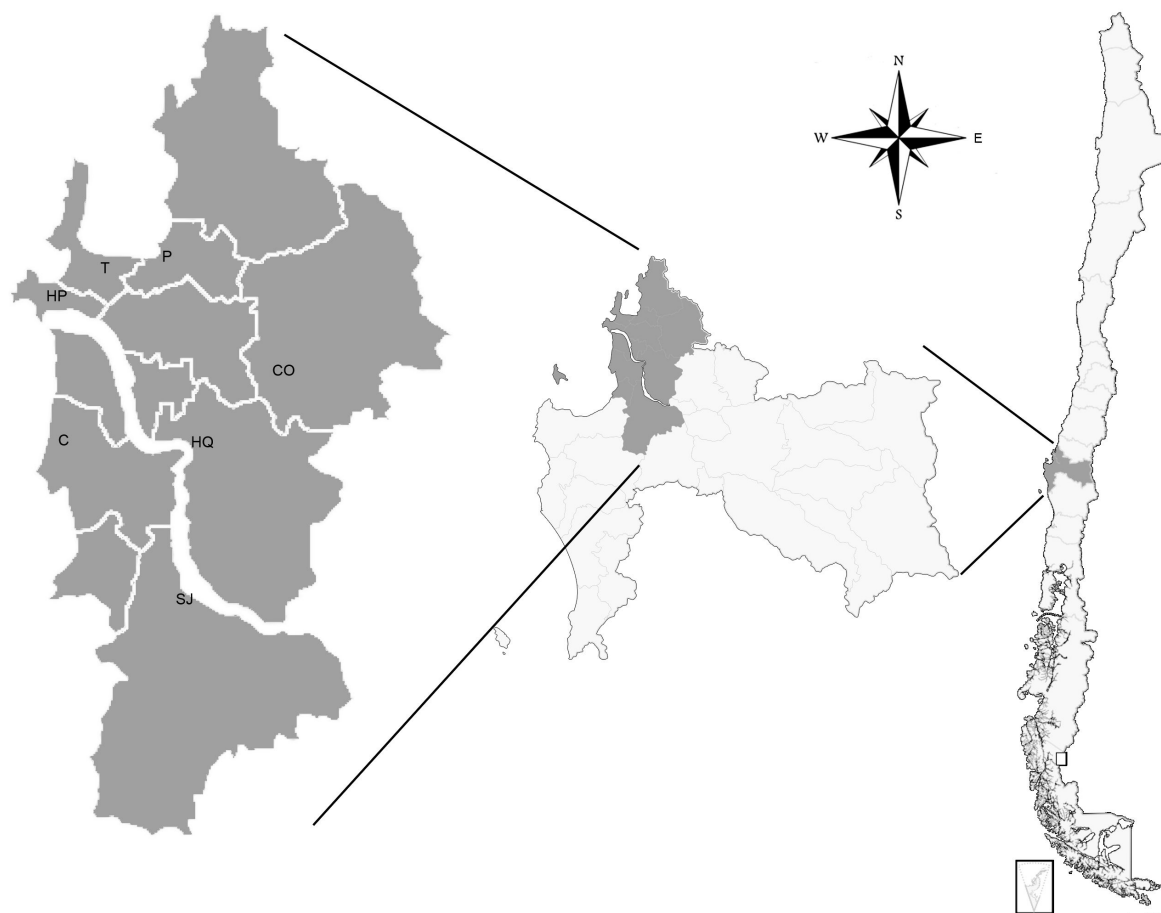


Figure 1. Geographical relation between the Concepción Province (left), the Biobío Region (center) and Chile (right), with the location where samples were obtained: T: Criaderos Talcahuano; HP: Criaderos Hualpén; C: Club de Rodeo Coronel; SJ: Medialuna Santa Juana; HQ: Medialuna Hualqui; P: Criadero Penco; CO: Medialuna Copiulemu.

of AMS III solution, three drops of Tween® 80, and 5 mL of ether. After that, the tube was centrifugated at 448 RCF for 2 minutes. The supernatant was discarded and the sediment was placed onto slides for light microscopy at 100X. The presence of at least one *F. hepatica* egg was considered to be a positive result. This technique has showed better sensitivity than other techniques for fecal examination of trematodes (Irie et al., 2015) and other intestinal parasites (Uga et al., 2002).

In order to compare the copro-prevalence between dichotomic variables, sex (male/female), age (0-6.9 years old [young]/7-24 [old]; given that 7 years old is the median age), body condition (0-2 [low]/3-5 [high]), Fisher exact tests were used. In addition, the confidence intervals are also given for examining the presence of intervals overlapping. The significance level was set at $P=0.05$. The confidence intervals (CI) are given for 95% confidence. Statistical analyses were performed using Stata 11/SE (StataCorp Ltd.).

The Ethics Committee of the Veterinary Sciences Faculty at the Universidad de Concepción approved and certified this study.

Fasciola hepatica eggs were found in the feces of ten of the 124 examined horses (8.06%, CI: 3.2-12.92), and in four localities: Club de Rodeo Coronel (5 infected horses; 12.82%; CI: 2.33-23.31%), Medialuna Hualqui (3; 15.00%; 0-30.65%), Criaderos Talcahuano (1; 16.67%; 0-46.49%) and Medialuna Santa Juana (1; 5.26%; 0-15.30%). The lower prevalence than in other species as ovine and caprine (Morales et al., 2000) can be explained by a possible lower susceptibility of horses to *F. hepatica* (Williams & Hodgkinson, 2017) or to a higher difficulty to find *Fasciola* eggs in horses' feces (Nelis et al., 2010). All horses were fed with pasture. There was not significant variation between parasite presence with any other analyzed variable.

Previous studies exploring the prevalence of *F. hepatica* in horses in the Maule Region (located 100–250 km north from the region examined in this study) reported a prevalence rate of 13.5% (Apt et al., 1993). The lower prevalence rate in our study can be explained by the fact that the El Maule Region is characterized by larger flat

lowlands, which encompass not only coastal plains, but also feature a larger proportion of the Central Valley and, extended to its eastern limit, the Los Andes mountain range, meaning that there is a greater likelihood that stagnant water is present. In addition, the treatment of horses against *F. hepatica* was not established through the country prior to that study. On the other hand, although the climate in the Concepcion province is typically more humid than that in the Central Valley, the climate has become drier along central Chile, so much so that it does not necessarily mean there is a greater likelihood of *F. hepatica* infection in Concepcion province than there was in the Central Valley two decades ago.

The prevalence of this infection in males was 6.33% (n=5. CI: 0.87-11.79%), while that of females was 11.11% (n=5. CI: 1.73-20.49%). The lack of a significant difference between sexes (Fisher test: P=0.49) is in agreement with the findings of previous studies performed in Chile (Apt et al., 1993; Muñoz et al., 2008). The sex bias in parasitism is usually explained by several hypotheses, as the behavioral, body mass, and hormonal/immunological differences between male and female hosts (Harrison et al., 2010; Kołodziej-Sobocińska, 2019). For instance, males of free-living mammal populations may be more prone to infection due to the fact that they move larger distances than females, enhancing the likelihood with which they come into contact with infecting parasites. The lack of a sex-biased parasitism in our study is expected, as the management of male and female horses does not differ; thus, both groups of horses may come into contact with infecting metacercariae, and both receive controlled food resources, reducing immunological differences.

The lack of an association between horse age and the presence of eggs (younger than 7 years old: 8.47%; CI: 1.24-15.71%. 7 years old and older: 7.69%; CI: 1.1-14.29%. Fisher test: P=0.56) is in agreement with the findings of a previous study conducted with thoroughbred horses (Alcaíno et al., 2005), and is contrary to the findings of previous studies that have explored both thoroughbred horses (Muñoz et al., 2008) and slaughtered horses (breeds not reported) (Apt et al., 1993), whereby higher prevalence rates of infection were reported in younger horses. A combination of factors, such as immunological status (favoring infection in younger animals) and length of exposure (with longer exposure times occurring for adult horses) may explain the lack of an association between a horse's age and the presence of *F. hepatica*. Nevertheless, new studies are necessary to determine the cause of this discrepancy.

Body condition was not significantly associated with the presence of *F. hepatica* eggs (Lower than 3: 11.11%; CI: 0-33.10%. Body condition 3 or higher: 7.82%; CI: 2.85-12.81%. Fisher test: P=0.54. See Table 1), as mean body condition scores of 3.12 and 3.0 were associated with negative (absence of eggs) and positive (presence of eggs) findings, respectively. In our study, we did not assess parasite abundance (the number of worms per host); however, the lack of an association between body condition and the presence of eggs suggests that the abundance was low, meaning that there was a low or null effect of body condition on infection status.

Table 1. Prevalence of fasciolosis in stabled Chilean breed horses in the province of Concepción, as associated with body condition (based on the Carroll and Huntington scoring system; n=124).

Body condition	Number of individuals (n)	Positive individuals (n)	Prevalence (%)
0	0	0	0
1	0	0	0
2	9	1	11.1
3	92	8	8.7
4	23	1	4.4
5	0	0	0

Further, only 10 horses were previously dewormed with triclabendazole; all of these horses belonged to the same farmyard, and this small number of dewormed horses may explain the lack of a significant association with parasitism (Not dewormed: 7.89% CI: 2.94-12.84%. Dewormed: 10%; CI: 0-28.59%. Fisher test: P=0.58). *Fasciola hepatica* eggs were present in the feces of one horse treated with triclabendazole. This might be due to a low medication dose, as this horse was not weighed prior to the triclabendazole treatment. Another possibility may be that *F. hepatica* was resistant to triclabendazole, which was previously reported in ruminants in localities near where this study was conducted (Romero et al., 2019).

In addition to the hypotheses exposed to explain the results, a common factor can explain the lacks of significant variation, the low number of positive individuals. The low prevalence makes difficult to find statistical association between variables, in such a way that those factors could affect the output, but we were not able to evidence it. On the other hand, the detection of eggs in feces in the horse is low sensitive, in such a way that the real prevalence of infection could be higher than the observed. Further studies, for instance detecting antibodies in serum, could enhance the sensibility and thus output different results, especially the estimation of prevalence rather than the findings of significant variation, given that the probability values were far from the significance level.

Finally, the results of this study suggest that Chilean breed horses constitute part of the reservoir of *F. hepatica* for both ruminant populations and humans in the Concepción province. However, the low prevalence rate of this infection raises the question of whether Chilean breed horses are really competent hosts for *F. hepatica*, releasing a number of eggs that is larger than the number of metacercariae they eat (amplification effect), or they are not a competent host, releasing fewer eggs than the metacercariae they eat (dilution effect).

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