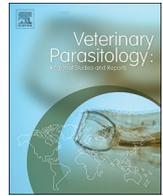




ELSEVIER

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Regional report

Prevalence of fasciolosis in slaughtered dairy cattle from São Miguel Island, Azores, Portugal

Ricardo Barbosa^{a,*}, Carlos Pinto^b, Patrícia Garcia^{a,c}, Armindo Rodrigues^{a,d}

^a FCT-UAç – Faculty of Sciences and Technology of the University of the Azores, Portugal

^b INIAV – National Institute of Agrarian and Veterinary Research, Portugal

^c cE3c/ABG – Centre for Ecology, Evolution and Environmental Changes/Azorean Biodiversity Group, Portugal

^d IVAR – Institute of Volcanology and Risk Assessment, Portugal

ARTICLE INFO

Keywords:

Fasciola hepatica
Dairy cattle
Azores
Fasciolosis
Risk areas

ABSTRACT

Fasciolosis, caused by the trematode *Fasciola hepatica*, is considered one of the most important parasitosis in dairy cattle due to significant economic losses. The main objectives of this study were to determine the prevalence of fasciolosis in dairy cattle slaughtered at São Miguel Island (SMI) slaughterhouse and to ascertain areas (parishes and dairy herds), within the island, with highest risk for fasciolosis. The association among cattle age and weight with the occurrence of fasciolosis was also determined. From a sample of 24,389 cattle slaughtered in two years (2015–2016), a total of 1134 dairy cattle livers were inspected and, during this period, 531 were positive to fasciolosis and 603 presented no typical fasciolosis lesions.

This study showed that fasciolosis has a broad geographical distribution on SMI and that is also found in cattle from other islands of the Azores archipelago, namely Flores Island. Although there were no significant differences in weight between healthy animals and parasitized ones, we observed that the older animals present a greater level of liver lesions and a higher level of parasitization, reflecting their longer period of exposure to the risk factor (ingestion of metacercariae in pastures infested by *Galba truncatula*).

1. Introduction

Fasciolosis is a helminthic zoonosis caused by the *Fasciola hepatica* trematode that can reach some taxa of animals, especially those used in the livestock industry, such as cattle, goats, sheep, swine and leporidae (Urquhart et al., 1998; Hurtrez-Boussès et al., 2001; Bowman, 2009; Mas-Coma et al., 2018). This disease also affects men, being a great concern for all those related to agriculture and public health (Furtado and Cunha, 2002; Mas-Coma et al., 2018).

In recent years, fasciolosis has become a significant public health concern due to the increasing number of human cases reported worldwide (Mas-Coma et al., 2009; Ashrafi et al., 2014; Cwiklinski et al., 2016; Mas-Coma et al., 2018). According to Cwiklinski et al. (2016), the World Health Organization (WHO) estimates that 17 million people are infected and a further 180 million are at risk of infection. *Fasciola* infections are limited to specific and typical geographical areas, especially where sheep and cattle are farmed intensively (Mas-Coma et al., 2005; Sripa et al., 2010; Mas-Coma et al., 2018).

This pathology is responsible for causing large annual economic losses in livestock producers and food industries worldwide, that can

reach €2.5 billion (Animal Health Ireland, 2013). In Europe, this parasitosis is particularly relevant in Ireland (Byrne et al., 2016), where about €90 million of economic losses are estimated for the Irish industries (Animal Health Ireland, 2013). Economic losses are usually associated to the decrease in meat and milk production (Sanchez-Vazquez and Lewis, 2013; Howell et al., 2015; Köstenberger et al., 2017). Depending on the infection level, fasciolosis can decrease by 20% the meat production in beef cattle, taking 80 days, after deworming, to reach meat production levels that are compatible with those of the marketplace. Also, it was shown that fasciolosis can reduce about 8% of milk production in dairy cattle (Animal Health Ireland, 2013). Economic losses are also associated with livers that are rejected for consumption in slaughterhouses (Khoramian et al., 2014; Innocent et al., 2017) that can reach rejection rates of 50%. In cattle, fasciolosis may also occasionally cause death, as it may trigger clostridial diseases (Animal Health Ireland, 2013; Khoramian et al., 2014).

São Miguel Island (SMI) is the largest of the nine islands of the Azores archipelago, located in the North Atlantic Ocean (36 to 39°N, 24 to 31°W). Its climate is oceanic, with 900 to 3000 mm of rainfall annually, and mild temperatures (17 °C in average, ranging from 9 to

* Corresponding author at: Universidade dos Açores, 9501-801, Portugal.

E-mail address: ricardobbarbosa91@gmail.com (R. Barbosa).

26 °C) (REOT-A, 2003; Brito, 2004; PRORURAL, 2007) with no frosts below 600 m altitude (Pinto et al., 2005). In addition to the climatic conditions, the nature of the soil and the existing grazing system of dairy cattle in the Azores generates favorable conditions for the development of some parasitosis (Mendes et al., 2016), such as fasciolosis.

According to the Council Regulation (EC) No 1760/2000 of 17 July 2000, establishing a system for the identification and registration of bovine animals (SNIRA, 2016), currently the total livestock on SMI is of 120,000 animals, of which 53,629 are dairy cows (SNIRA, 2016). Most of these dairy cows are from the Holstein-Friesian breed, distributed over 1362 dairy herds (POSEI, 2016), with an average of 39 cows per herd (SNIRA, 2016). Taking advantage of the good grazing conditions, the dairy cattle industry became one of the most important economic activities in the Azores (Mendes et al., 2016). Also, in this archipelago, dairy cattle live under grazing conditions throughout the year and present the highest rates of longevity, unlike other types of cattle categories like yearling and heifers, most of them from Holstein-Friesian breed, which are slaughtered before two years old.

The last study regarding the fasciolosis status on SMI was carried in 1998 (Furtado and Cunha, 2002), therefore it is necessary to update its current prevalence throughout the island. Since fasciolosis lesions can be detected macroscopically in the bile ducts of the liver (Cunha, 1991; Taylor et al., 2007; Khoramian et al., 2014), the objectives of this study were to determine the prevalence of fasciolosis in dairy cattle slaughtered at SMI slaughterhouse and to ascertain the areas (parishes and dairy herds), within the island, with the highest prevalence of fasciolosis. The association between cattle age and weight with the occurrence of fasciolosis was also determined. Also, a comparison was made between macroscopically parasitized animals by fasciolosis *versus* non-parasitized, in terms of weight, age and liver lesions level. Positive cases of fasciolosis included all livers that were infected with liver flukes, with or without the typical lesions of this parasitosis.

2. Materials and methods

2.1. Anatomopathological observations of dairy cow's livers

The analysis was conducted through anatomopathological observation of the fasciolosis characteristic liver lesions from dairy cattle slaughtered in SMI slaughterhouse. The data was collected from January of 2015 to December of 2016. The procedure consisted in the identification of the previously slaughtered animals (slaughterhouse order number), followed by liver inspections. Liver inspection or liver examination includes the observation of parietal and visceral surfaces and incising parts around bile ducts, to identify liver flukes or the typical lesions of fasciolosis. For each animal, the following data was obtained: order number, earring number, age (in months) and cold carcass weight. From a sample of 24,389 cattle slaughtered in these two years, a total of 1134 dairy cattle livers were inspected and, during this period, 531 were positive to fasciolosis and 603 presented no typical fasciolosis lesions.

From this dataset, the 531 livers that were positively diagnosed for fasciolosis were used to assess liver lesion levels. The liver lesion level was based on a qualitative assessment, considering the observation of the entire surface of the liver, attributing the lesion level according to

Table 1
Grading of the lesions by fasciolosis.

Lesion grade	Lesion description
Absent	Normal liver
Mild	Slightly thickened and calcified bile ducts in the caudal half of the visceral face; mild sclerosis/fibrosis in the caudal half; slight atrophy of the caudal half
Moderate	Thickened and calcified bile ducts in the caudal half of the visceral face; sclerosis/fibrosis in the caudal half; considerable atrophy of the caudal half; possible agglomeration of calcified flukes in the bile ducts; possible formation of abscesses
Intense	Thickened and calcified bile ducts in the caudal half of the visceral face; evident sclerosis/fibrosis in the caudal half; complete atrophy and reduction of the caudal half; possible agglomeration of calcified flukes in the bile ducts; possible formation of abscesses

Table 2
Grading of parasitism by fasciolosis.

Parasitism grade	Description
Level 0	Without parasites.
Level 1	Presence of 1 to 3 parasites.
Level 2	Presence of 4 to 6 parasites.
Level 3	Presence of a number equal to or > 7 parasites.

the description in Table 1. The liver was also qualitatively classified according to the level of parasitosis, which consisted in the number of parasites (flukes) present in the liver, according to the categorization presented in Table 2.

2.2. Prevalence and geographical distribution of the fasciolosis positive diagnosed cases

To determine the geographic distribution of the fasciolosis positive cases, data such as the location of the dairy herd (considered as the location where the animal spent more time or at least 6 months), was gathered from the databases SNIRA (National System of Animal Registration and Information – Sistema Nacional de Informação e Registo Animal) and SIARA (Information System for Agricultural Holdings of the Autonomous Region of the Azores – Sistema de Informação das Explorações Agropecuárias da Região Autónoma dos Açores). Data such as birth place and cattle movement were used and gathered also from the databases SNIRA and SIARA. Both databases comprised information regarding 11,452 animals (for the year of 2015) and 12,937 animals (for the year of 2016).

Table 3 represents data on the number of dairy herds and dairy cows eligible for the POSEI (Council Regulation (CE) No 247/2006 of January 2006, laying down specific measures for agriculture in the outermost regions of the European Union) dairy cow program at SMI, for the years 2015 and 2016. In these two years, from a sample of 24,389 slaughtered dairy cows, 531 were positive for fasciolosis (a mean of 266 per year), corresponding to a mean prevalence of 2.2%. Within a total of 1393 dairy herds from SMI, 151 (10.8%) had at least one recorded case of fasciolosis. There were no missing data in this study, as all data for every animal were complete.

The prevalence rates were compared with the geographic areas where the animal spent most of their life, through the elaboration of Geographic Information System cartograms, to relate the occurrence of fasciolosis with the altimetric and pluviometric variables. Cartograms were obtained using the software's GIS and ArcGIS Desktop-ArcMAP.

2.3. Data management and analysis

The association between the liver lesion level and the age or the weight of the animal was estimated through Spearman correlations, as well as the association between the liver lesion level and the level of parasitosis. The weight of the animals, their age and the weight of the livers were analyzed according to the liver lesion level, using an analysis of variance, one-way ANOVA; whenever the ANOVA test was significant, Tukey tests were performed to identify differences between data sets. All statistical analyzes were performed in the SPSS® V21.0 for

Table 3

Total number of dairy cows, number of slaughtered dairy cows, number and percentage of cows with fasciolosis compatible lesions, number and percentage of dairy herds with at least one diagnosed case of fasciolosis, for São Miguel Island in the years of 2015 and 2016 ($P \leq .005$) (Source: São Miguel Island slaughterhouse Inspection Service; SNIRA; POSEI).

Year	Dairy cows	Slaughtered dairy cows	Dairy cows with fasciolosis (%)	Number of dairy herds	Number of dairy herds with fasciolosis (%)
2015	51,590	11,452	185 (1.6)	1424	121 (8.5)
2016	53,629	12,937	346 (2.7)	1362	180 (13.2)
Total		24,389	531 (2.2)		
Mean				1393	151 (10.8)

Windows program, with a statistical significance level of $P < .05$.

3. Results

In total, the livers of 1134 dairy cattle were inspected, of which 1119 were from SMI and the remaining 15 were from other islands of the archipelago (14 were from Flores Island and 1 from São Jorge Island).

Results showed that the animals without liver lesions were significantly younger, with a mean age of 56.8 months, when compared to the other group of animals with mild liver lesion level (aged over 86 months), moderate lesion level (with 92 months) and intense lesion level (with 100 months) (one-way ANOVA, $df(31126) = 156.366$, $P \leq .001$). There was also a positive and moderate association between the age of the animals and the liver lesion level ($r_s(1130) = 0.552$, $P \leq .001$), revealing that the liver lesion level increases with the age of the animal (Fig. 1).

No significant differences were observed for the mean weight of the animals regarding the liver lesion level (one-way ANOVA, $df(31126) = 1.143$, $P = .331$). A positive association was found between the liver lesion level and the level of parasitism by *F. hepatica* (i.e., the number of parasites present in the liver of the animals) ($r_s(653) = 0.176$, $P \leq .001$), revealing that the higher the level of the liver lesion, the greater the probability of occurrence of the *F. hepatica* parasites in the liver. No significant effect of the liver lesion level in the livers weight of the animals was observed (one-way ANOVA, $df(2,57) = 1749$, $P = .183$).

The geographic distribution of the observed fasciolosis positive cases by the 59 parishes of SMI in 2015 and 2016 (Fig. 2) revealed that this parasitosis is predominantly confined to the eastern half of the island.

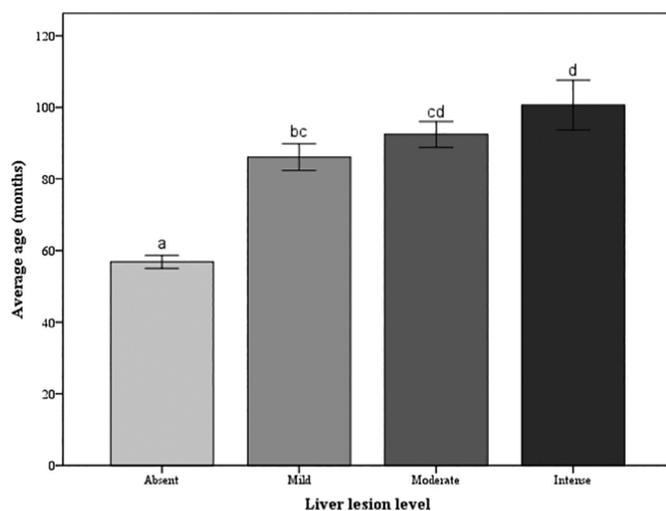


Fig. 1. Age (mean \pm SE) of the animals observed in slaughterhouse according to the liver lesion level. Locations with different letters are significantly at $P = .05$ (Tukey-HSD tests).

4. Discussion

In SMI, Azores, most of the pastures allow a permanent grazing regime throughout all the year, except in winter at the altitudes above 600 m, and in summer, at the altitudes below 200 m (Pinto et al., 2005). Associated with this permanent grazing regime, the mild temperatures and the strong annual rainfall, as well as the soggy soils for most of the year, create favorable conditions for the development of the *lymnaeid* species, intermediate hosts of *F. hepatica*. Also, in the Azores, the dairy herds are constantly grazing in the open air, which combined with the fact that dairy cows are the group of cattle with the highest longevity rate, makes them favored hosts for the parasite *F. hepatica* (Furtado, 2000).

To estimate the prevalence of fasciolosis in the parishes of SMI and in its dairy herds, we used as a selection criterion the dairy herd where the animal spent most of its life, which does not invalidate the fact that an animal has been contaminated in another dairy herd where it has spent less time. Not all the diagnosed fasciolosis cases had the presence of the *F. hepatica* parasite, indicating that these animals had been previously infected by the parasite and therefore have the characteristic fasciolosis liver lesions.

The annual data on the number of dairy cows and SMI dairy herds (Table 3) shows a fasciolosis infection rate for the years of 2015 and 2016 of 1.6% and 2.7%, respectively. The percentage of farms with at least one diagnosed case of fasciolosis in SMI for the years of 2015 and 2016, were, respectively, 8.5% and 13.2%. These data reveal an increase from 2015 to 2016 for both the fasciolosis infection rate and the total of infected dairy herds.

Regarding the data from the 15 dairy cattle outside of SMI, 14 were from Flores Island and positive to fasciolosis, and 1 animal was from São Jorge Island, but not positive to fasciolosis. Concerning the cases from Flores Island, it is noteworthy that all these animals have lived their lifetime on this island, which means that fasciolosis is not limited to SMI, contrary to what was stated in some previous studies (Mendonça, 1987; Furtado, 2000).

The longer the longevity of a bovine, the greater is its exposure to risk factors for fasciolosis, namely the direct contact with the parasite and the consequent development of fasciolosis sequelae (Innocent et al., 2017). Thus, the positive association observed between the liver lesion level and the mean age was expected; the animals with healthy livers (absent lesions) were much younger than the animals with livers that were rejected for consumption due to the positive diagnosis of fasciolosis, i.e., livers with a mild, moderate and intense lesion levels.

Regarding the relationship between the lesion level and the animal mean weight, no significant differences were found between bovines with no lesion of fasciolosis and those with fasciolosis. These results are in line with those from a previous study by Furtado and Cunha (2002) which reported a rate of infection of fasciolosis of 7.6% in the year of 1998. Although not significantly different, in the current study, the animals with intense liver lesions presented a relatively lower mean weight, compared to the other liver lesion groups (absent, mild and moderate lesions). Such lower weight could be explained by the simple fact that animals with intense lesions are, in average, older than the others.

In what concerns to the association between the liver lesion level

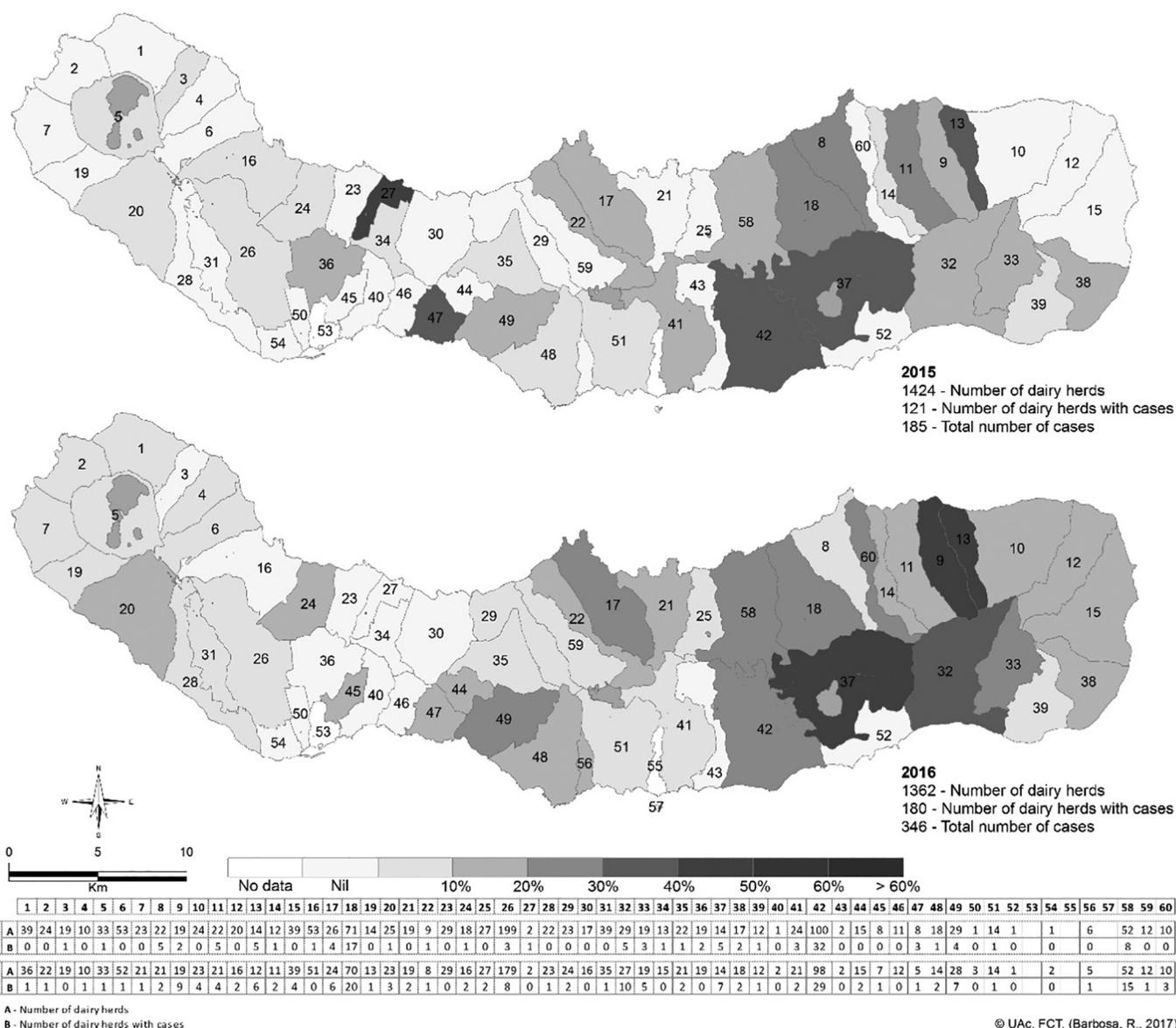


Fig. 2. Geographic distribution within the 59 parishes of São Miguel Island of fasciolosis positive cases observed in São Miguel Island slaughterhouse, in 2015 and 2016.

and the presence of the parasite *F. hepatica* in the liver, the results revealed that the higher the lesion level of the liver, the greater the probability of identifying one or more parasites.

The association between the liver lesion level and the livers weight of the animals presented no significant differences. However, it was found that 50% of the animals with a mild lesion level had a liver weighing > 10.5 kg, while in the remaining groups (without lesion or moderate lesion) 75% of the livers were below this value. This apparent difference between the weight of the livers with mild lesion level, absent lesion level and moderate lesion level may result from the fact that the group of animals with mild liver lesion level is at the peak of their productive cycle. Moreover, animals with a moderate lesion level in the liver correspond to the group that is already entering the end of their productive life, which along with external factors to fasciolosis will influence the weight of the liver.

The geographical distribution of fasciolosis in SMI reveals that fasciolosis is more prevalent on the eastern half of the island, a result that is in line with the results from the study carried out by Martins in 1991. According to this author, the occurrence of fasciolosis in this part of the island is closely linked to the presence of the intermediate host, the

species *Galba truncatula*, which is confined to this area. However, for the years 2015 and 2016 there were some parishes in the western half of SMI where the presence of dairy herds with fasciolosis was recorded. This implies that transmission has occurred in the western part of the island.

During the study of these fasciolosis cases, it was possible to observe that there were some animals from the western part of the island with the presence of flukes in their liver. Since these same animals lived all their lifetime on the SMI western half, the question of the geographic distribution of its intermediate host was relaunched, and the question of the possible existence of other *lymnaeid* species that could also act as an alternative intermediate host was also raised, as previously hypothesized by Teixeira et al. (2012).

The most affected parishes by fasciolosis are located at a higher altitude (> 200 m), corresponding to areas of permanent grazing regimes and where the highest rainfall rates are observed (Figs. 2, 3 and 4). In the eastern part of SMI, areas with the highest prevalence of fasciolosis overlap with the areas with greater altitude and rainfall, as can be inferred from the comparison among Figs. 2, 3 and 4.

In fact, the occurrence of fasciolosis cases are closely linked to the

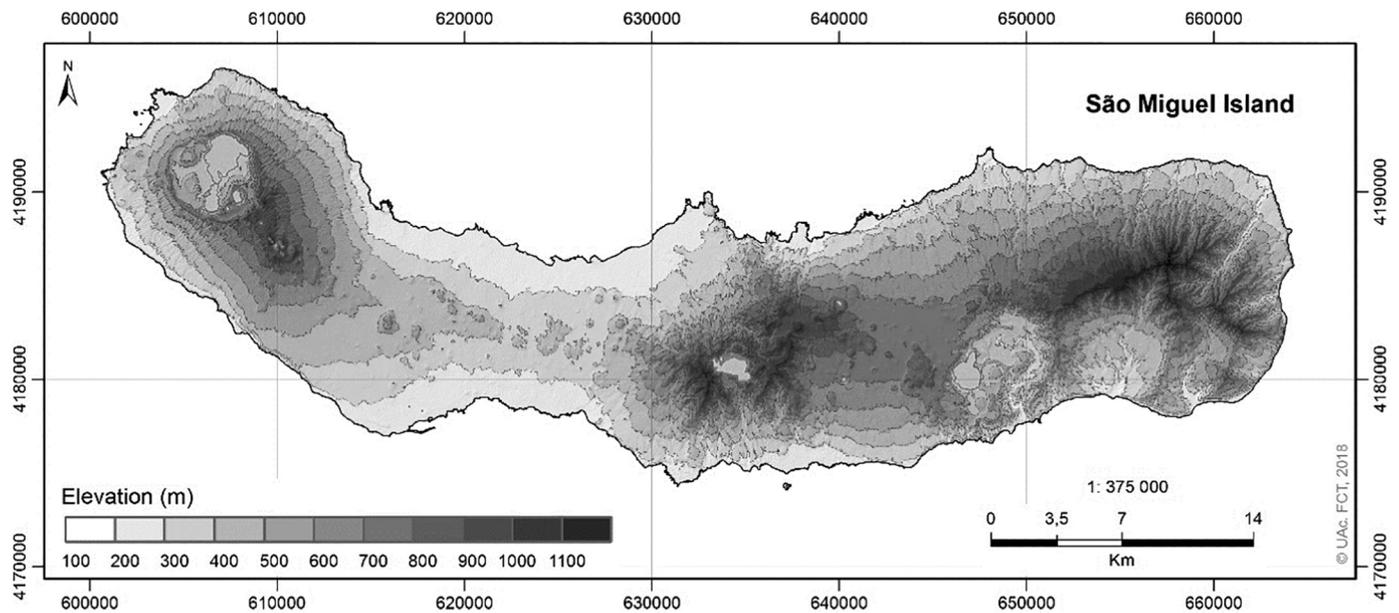


Fig. 3. Elevation map of São Miguel Island (Source: Geography Section of the Faculty of Sciences and Technology of the University of the Azores, 2019).

climatic factors in SMI, which influence the viability of *F. hepatica*, as well as of its intermediate host, *G. truncatula* (Furtado, 2000). All the affected areas strongly indicate the influence of rainfall. Pasture land located at high altitude areas with high rainfall, both in summer and winter, present a higher density of grazing dairy cattle, and therefore are the most affected by fasciolosis. In the geographical areas of the western side of the island, especially in areas of low altitude (< 200 m) and very low rainfall, cases of fasciolosis are practically nonexistent. However, the importation of infected cattle, the purchase of forage bales with cystic metacercariae, or even its accidental transport by anthropic action, can thus enable the occurrence of cases of this parasitosis in western half of the island. Yet, the issues related to the distribution of the known intermediate host for the island, *G. truncatula*, or the possible existence of another intermediate host, remains to be clarified.

5. Conclusions

The epidemiological characterization of the fasciolosis positive cases observed in SMI Slaughterhouse, referring to SMI dairy herds, confirmed that this parasitosis is still well disseminated in the eastern part of the island. However, this study revealed that this parasitosis already tends to expand to the western half, where, according to previous studies, the existence of the intermediate host of *F. hepatica*, *G. truncatula*, has never been observed. This study, in addition to showing that fasciolosis has a wider distribution on SMI, also reveals that this parasitosis already affects other islands from the Azores archipelago, namely Flores Island.

The rate of infection and dairy herd percentage with fasciolosis in both the years of 2015 and 2016, was of 2.2% and 10.8%, respectively. There were no significant differences between the weight of animals without fasciolosis lesions and parasitized animals, which does not render into economic losses regarding meat production, but it can

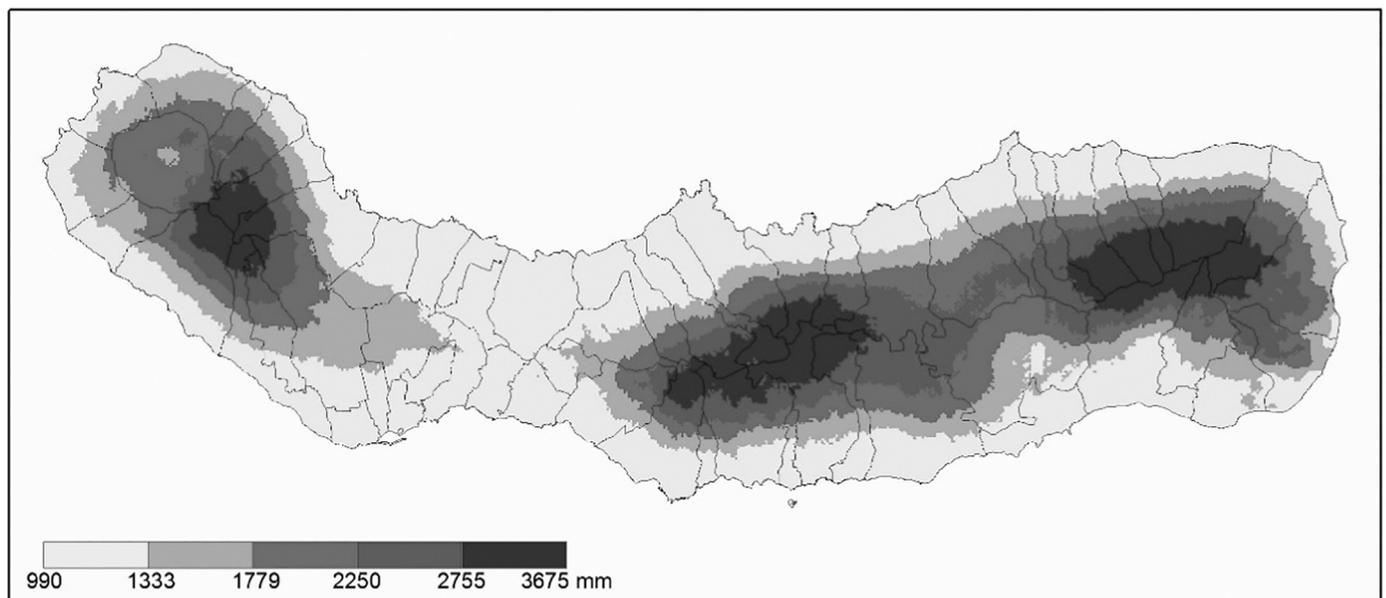


Fig. 4. Rainfall map of São Miguel Island (Source: Geography Section of the Faculty of Sciences and Technology of the University of the Azores, 2016).

translate into economic losses for the farmer in terms of milk production. The same was seen regarding the weight of the livers from the animals observed in the slaughterhouse, where no significant differences were found between animals without fasciolosis lesions and those with fasciolosis. An ideal study design would be the comparison of weight losses in parasitized animals *versus* healthy animals in a controlled environment and using animals within the same age group, which would allow a better evaluation of the possible economic impact of this parasitosis. Another interesting approach would be the study of the fasciolosis influence on the milk production in the Azores, since it is known that the economic impact of this parasitosis can be highly relevant.

Ethical statement

The study entailed recording of the normal meat inspection process of animals sent to slaughter for human consumption. No ethical approval was required nor sought as no alteration was required to the normal processing of these animals.

Declaration of Competing Interest

We declare that we have no conflict of interest.

Acknowledgments

The authors thank São Miguel Agrarian Development Service and São Miguel Island slaughterhouse for allowing and supporting this study. The authors also thank António Medeiros and the Geography Section of the Faculty of Science and Technology of the University of the Azores for the technical input in elaborating the cartograms.

References

- Animal Health Ireland, 2013. Liver Fluke – the Facts. In: Parasite Control Leaflet Series, Vol. 5, Ver. 2, Retrieved from Animal Health Ireland. <https://online.flippingbook.com/view/128755/1/> (September 12, 2016).
- Ashrafi, K., Bargues, M.D., O'Neill, S., Mas-Coma, S., 2014. Fascioliasis: a worldwide parasitic disease of importance in travel medicine. *Travel Med. Infect. Dis.* 12 (6), 636–649. <https://doi.org/10.1016/j.tmaid.2014.09.006>.
- Bowman, D.D., 2009. Helminths. In: Bowman, D.D. (Ed.), *Georgis' Parasitology for Veterinarians*, 9th ed. Saunders – Elsevier, St. Louis, Missouri, USA, pp. 115–124.
- Brito, R.S., 2004. São Miguel a Ilha Verde – Estudo geográfico (1950–2000). Ponta Delgada: Fábrica de Tabaco Micaelense, SA; COINGRA – Companhia Gráfica dos Açores, Lda; EDA – Empresa de Electricidade dos Açores, SA; Universidade dos Açores. pp. 381.
- Byrne, A.W., McBride, S., Lahuerta-Marin, A., Guelbenzu, M., McNair, J., Skuce, R.A., McDowell, S.W.J., 2016. Liver fluke (*Fasciola hepatica*) infection in cattle in Northern Ireland: a large-scale epidemiological investigation utilising surveillance data. *Parasite Vector.* 9 (1), 209. <https://doi.org/10.1186/s13071-016-1489-2>.
- Cunha, R.T., 1991. Predação em *Lymnaea truncatula* (Müller) por *Oxychilus atlanticus* (Morelet & Drouët). In: Trabalho de síntese elaborado no âmbito das provas de Aptidão Pedagógica e Capacidade Científica. Universidade dos Açores, Ponta Delgada, pp. 105.
- Cwiklinski, K., O'Neill, S.M., Donnelly, S., Dalton, J.P., 2016. A prospective view of animal and human Fasciolosis. *Parasite Immunol.* 38 (9), 558–568. <https://doi.org/10.1111/pim.12343>.
- Furtado, R.M.M., 2000. Infestação por *Fasciola hepatica* (Linnaeus) do gado bovino da ilha de São Miguel. Trabalho de licenciatura em Biologia. Universidade dos Açores, Ponta Delgada, pp. 122.
- Furtado, R.M.M., Cunha, R.T., 2002. Fasciolose bovina no Matadouro Frigorífico e Industrial de Ponta Delgada, São Miguel (Açores): Atualização da taxa de infestação. *Açoreana* 9 (4), 331–341.
- Howell, A., Baylis, M., Smith, R., Pinchbeck, G., Williams, D., 2015. Epidemiology and impact of *Fasciola hepatica* exposure in high-yielding dairy herds. *Prev. Vet. Med.* 121 (1–2), 41–48. <https://doi.org/10.1016/j.pvetmed.2015.05.013>.
- Hurtrez-Boussès, S., Meunier, C., Durand, P., Renaud, F., 2001. Dynamics of host-parasite interactions: the example of population biology of the liver fluke (*Fasciola hepatica*). *Microbes Infect.* 3 (10), 841–849. [https://doi.org/10.1016/S1286-4579\(01\)01442-3](https://doi.org/10.1016/S1286-4579(01)01442-3).
- Innocent, G.T., Gilbert, L., Jones, E.O., McLeod, J.E., Gunn, G., McKendrick, I.J., Albon, S.D., 2017. Combining slaughterhouse surveillance data with cattle tracing scheme and environmental data to quantify environmental risk factors for liver fluke in cattle. *Front. Vet. Sci.* 4, 65. <https://doi.org/10.3389/fvets.2017.00065>.
- Khoramian, H., Arbabi, M., Osqoi, M.M., Delavari, M., Hooshyar, H., Asgari, M., 2014. Prevalence of ruminants fascioliasis and their economic effects in Kashan, center of Iran. *Asian Pac. J. Trop. Biomed.* 4 (11), 918–922. <https://doi.org/10.12980/APJTB.4.2014APJTB-2014-0157>.
- Köstenberger, K., Tichy, A., Bauer, K., Pless, P., Wittek, T., 2017. Associations between fasciolosis and milk production, and the impact of anthelmintic treatment in dairy herds. *Parasitol. Res.* 116 (7), 1981–1987. <https://doi.org/10.1007/s00436-017-5481-3>.
- Martins, A.M.F., 1991. Distribuição dos moluscos de água doce em São Miguel e na Terceira. *Açoreana* 7 (2), 257–276.
- Mas-Coma, S., Bargues, M.D., Valero, M.A., 2005. Fascioliasis and other plant-borne trematode zoonoses. *Int. J. Parasitol.* 35 (11–12), 1255–1278. <https://doi.org/10.1016/j.ijpara.2005.07.010>.
- Mas-Coma, S., Valero, M.A., Bargues, M.D., 2009. Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. *Adv. Parasitol.* 69, 41–146. [https://doi.org/10.1016/S0065-308X\(09\)69002-3](https://doi.org/10.1016/S0065-308X(09)69002-3).
- Mas-Coma, S., Bargues, M.D., Valero, M.A., 2018. Human fascioliasis infection sources, their diversity, incidence factors, analytical methods and prevention measures. *Parasitology* 145 (13), 1665–1699. <https://doi.org/10.1017/S0031182018000914>.
- Mendes, J., Pinto, C., Bernardo, S., Lima, J.R., Maia, C., 2016. Prevalence of *Ostertagia ostertagi* lesions in slaughtered dairy cattle from São Miguel Island, Azores, Portugal. *Vet. Parasitol. Reg. Stud. Reports* 3, 60–65. <https://doi.org/10.1016/j.vprsr.2016.06.003>.
- Mendonça, M.M., 1987. A fasciolose por *Fasciola hepatica* L., 1758 em S. Miguel (Açores). Contribuição para o estudo das condições ecológicas da sua transmissibilidade. Dissertação para a candidatura a categoria de Investigador Auxiliar. Instituto de Investigação Científica Tropical, Lisboa, pp. 366.
- Pinto, C., Santos, V.M., Dinis, J., Peleteiro, M.C., Fitzgerald, J.M., Hawkes, A.D., Smith, B.L., 2005. Pithomycototoxicosis (facial eczema) in ruminants in the Azores, Portugal. *Vet. Rec.* 157 (25), 805–810.
- POSEI, 2016. Açores, Portugal. Retrieved from Governo Regional dos Açores-. <http://posei.azores.gov.pt/ficheiros/legislacao/1592008161220.pdf> (January 22, 2017).
- PRORURAL, 2007. Programa de Desenvolvimento Rural da Região Autónoma dos Açores 2007–2013. Retrieved from Governo Regional dos Açores. <http://proruralmais.azores.gov.pt/> (January 22, 2017).
- REOT-A, 2003. *Relatório do Estado do Ordenamento do Território*. Secretaria Regional do Ambiente e do Mar. vol. 318.
- SNIRA, 2016. Sistema Nacional de Informação e Registo Animal. Instituto de Financiamento da Agricultura e Pescas. Portugal. Retrieved from. <https://www.ifap.pt/> (January 22, 2017).
- Sripa, B., Kaewkes, S., Intapan, P.M., Maleewong, W., Brindley, P.J., 2010. Food-borne trematodiasis in Southeast Asia: epidemiology, pathology, clinical manifestation and control. In: *Advances in Parasitology*. vol. 72. Academic Press, pp. 305–350. [https://doi.org/10.1016/S0065-308X\(10\)72011-X](https://doi.org/10.1016/S0065-308X(10)72011-X).
- Taylor, M.A., Coop, R.L., Wall, R.L., 2007. Parasites of the liver. In: Taylor, M.A., Coop, R.L., Wall, R.L. (Eds.), *Veterinary Parasitology*, 3rd ed. Blackwell Publishing, Oxford, pp. 244–251.
- Teixeira, T., Rosa, J.S., Rainha, N., Baptista, J., Rodrigues, A., 2012. Assessment of molluscicidal activity of essential oils from five Azorean plants against *Radix peregra* (Müller, 1774). *Chemosphere* 87 (1), 1–6. <https://doi.org/10.1016/j.chemosphere.2011.11.027>.
- Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W., 1998. *Helminthologia veterinária*. In: Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W. (Eds.), *Parasitologia veterinária*, 2ª ed. Guanabara-Koogan S.A, Rio de Janeiro, pp. 88–98.
- Vazquez, M.J.S., Lewis, F.I., 2013. Investigating the impact of fasciolosis on cattle carcass performance. *Vet. Parasitol.* 193, 307–311. <https://doi.org/10.1016/j.vetpar.2012.11.030>.