DISTRIBUTION OF *FASCIOLA SPP*, *DICROCOELIUM DENDRITICUM* AND *CALICOPHORON DAUBNEYI* IN SMALL RUMINANTS IN THE MEDITERRANEAN BASIN: A SYSTEMATIC REVIEW

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Abstract

The most common flukes found in sheep in Europe, Asia and Africa are the liver flukes Fasciola spp., Dicrocoelium dentriticum and the rumen fluke Calicophoron daubneyi. The prevalence of F. hepatica in Europe varies among countries (from 47.3 % in Greece, 57.4 % in Spain, and 95% in Italy). Several studies have been conducted in Africa and Asia as concern the prevalence of Fasciola in sheep but limited studies have been done in goats. The prevalence of D. dentriticum in sheep has been reported to be 6.7-86.2% in Italy and to range from 0.2%- to 70% in Greece. The prevalence of D. dentriticum in sheep was found to be 5% in Egypt and 3,85 to 23,55% in Turkey. Only three surveys have been conducted to measure the prevalence of D. dentriticum in goats in the Mediterranean basin indicating a variation from 0.9% to 42.42 %. C. daubneyi is the dominant rumen fluke species in Europe with significant clinical importance in ruminants. A positive correlation between the prevalence of F. hepatica and C. daubneyi has been observed, as well as a similarity in some potential risk factors (vegetation and rainfall variables) for both parasites. There are very few studies about the epidemiology of C. daubneyi in sheep and goats in the Mediterranean area. Rumen fluke should now be considered as an emerging parasitic infection of ruminant livestock in Europe, with a significant increase in the number of free rediae and a high prevalence of infection observed in small ruminants in many countries in the Mediterranean basin. The best approach to restrict the occurrence of diseases caused by flukes in this basin is to implement forms of integrated parasite control based on proper husbandry practices and strategic treatments.

Keywords: *flukes; epidemiology; emerging parasitic infection; sheep; goa*

Introduction

Sheep and goat farming has a prominent role in the economy of Europe, as the small ruminant population is more than 100 million heads. However, small ruminant farming is influenced by several factors such as breeding methods, infectious diseases and parasitic diseases (Park and Haenlein, 2006).

Concerning sheep and goats populations in Africa, East and West Africa topped the producers with more than 200 million small ruminants in 2011 (Jahnke et al., 1987) and with more than 10 indigenous sheep breeds (Lahlou-Kassi, 1986). In southern Europe, the total sheep population is estimated at 41,33 millions, of which 16,64 millions are dairy animals. As for goats, the population reaches the number of 10,26 millions. In Turkey, the sheep and goats populations are 30,98 and 10,35 million respectively (Eurostat, 2017).

The most common flukes found in sheep in Europe (Cringoli et al., 2002; Cringoli et al., 2004), Asia and Africa (Morsy et al., 2005; Gargilli et al., 1999; Mazyad et al., 2002) are the liver flukes *Fasciola hepatica, Dicrocoelium dentriticum* and the rumen fluke *Calicophoron* (*Paramphistomum*) *daubneyi*.

In the past 50 years, environmental changes caused by humans have contributed to the spread of diseases of domestic animals caused by flukes, with *Fasciola* and *Paramphistomum* in the wet and moist environments (building of dams, new irrigation systems), and *Dicrocoelium* in desertified tropical and subtropical areas (Otranto and Traversa, 2003).

Fasciola spp., *D. dentriticum* and *C. daubneyi* are trematode parasites found throughout Europe and Africa which affects a range of hosts, including ruminants, horses, wild animals such as deer, rabbits and hares, and humans. Loss of production associated with *Fasciola* infection and overt clinical disease results in significant financial loss in the global farming industry, the costs being estimated at over $3 billion per year (Spithill et al., 1999). For the production loss associated with *D. dentriticum* and *C. daubneyi* data are very scant.

This review will be conducted in line with the PRISMA statement 2009 (Moher et al., 2009). Articles will be selected for inclusion into the systematic review via the identification of all potentially relevant citations through the search strategy. The citations within identified articles will also be included in the screening process. Duplicates will be excluded, followed by screening of titles and abstracts with articles excluded if they do not explicitly report occurrence or prevalence of *Fasciola* spp., *D. dendriticum* and *C. daubneyi*. Data from the Northern Africa, Western Asia and Northern Europe between 1990 - 1st September 2018 will be evaluated and encapsulated. Paper with English abstract will also be included.

Data in this study is contemplated to expand current understanding about geographic distribution of infections with *Fasciola* spp., *D. dendriticum* and *C. daubneyi* in small ruminants in the Mediterranean basin. This information will be helpful for the design and implementation of more effective control measurements against *Fasciola* spp., *D. dendriticum* and *C. daubneyi.*

***Fasciola spp*. infection in small ruminants in the Mediterranean basin**

Fasciolosis is prevalent in many parts of Europe (Table1) and is one of the important helminthic infections in small ruminants. Fasciolosis is caused by *F. hepatica* in temperate zones of Europe, Africa Americas and Australia and by *F. gigantica* in tropical zones of Africa and Asia. Both species overlap in occurrence in subtropical areas (Mas-Coma et al., 2005; Amer et al., 2011). *Fasciola* spp require a snail to complete the life cycle and are found in wet lowland or mountain pastures.

Table 1. Prevalence (%) of fasciolosis in small ruminants in the Mediterranean basin.

Country	Year of study	Diagnostic method	*Fasciola* spp				References
			Sheep		Goats		
			N	%	N	%	
Spain	1995-2006	PM	-	-	1885	0.53	Alasaad et al. 2008
Spain	1995-2006	FE	-	-	267	1.87	Alassad et al. 2008
Spain	NS	ELISA	47(F)	57.4	-	-	Perez-Creo et al. 2016
Spain	NS	ELISA	603	22.7	-	-	Perez-Creo et al. 2016
Italy	2004-2005	FLOTAC	121(F)	12.40	-	-	Musella et al. 2011
Italy	2000-2013	FE	150	81-95	-	-	Bosco et al. 2015
Italy	2001	FE	-	-	-	-	Sanchez-Andrade et al. 2003
Italy	1999-2000	FE	788	2.3	-	-	Cringoli et al. 2002
Italy	2012-2013	FLOTAC	89(F)	7.9	-	-	Rinaldi et al. 2015
Greece	1999	PM	4273	0.1	1292	0.2	Theodoropoulos et al. 2002
Greece	NS	PM	254	15.75	-	-	Katsoulos et al. 2011
Greece	NS	FE	152	0.3-2.7	-	-	Mavrogianni et al. 2014
Greece	2006-2007	FE	557	0.5	-	-	Kantzoura et al. 2012
Greece	2006-2007	Copro-antigen	346	39	234	9	Kantzoura et al. 2011
Greece	2006-2007	ELISA	499	47.3	372	15.9	Kantzoura et al. 2011
Tunisia	2004-2005	ELISA	20	35	19	68.4	Hammami et al. 2007
Algeria	1994-2001	ELISA	463	6.4-23.5	-	-	Mekroud et al. 2004
Algeria	1994-2001	FE	3541	8.5-18.2	-	-	Mekroud et al. 2004
Egypt	NS	NS	NS	12.7	-	-	Mazyad et al. 2002
Egypt	NS	FE	400	3.25-15.75	-	-	El-Shazly et al. 2005
Egypt	NS	FE	180	20.56	-	-	El-Shazly et al. 2005
Egypt	NS	FE	50	30	-	-	Haridy et al. 2006
Egypt	2012-2014	PM	2058	14.7	-	-	Amer et al. 2016
Egypt	NS	FE	NS	40	-	-	Morsy et al. 2005
Egypt	NS	FE	409	17.84	185	5.40	El-Shazly et al. 2002
Egypt	NS	FE	29	17.2	-	-	Abdel-Nasser A.H. and Refaat M.A.K, 2010
Turkey	NS	NS	NS	26	-	-	Abu Zinada et al. 1999
Turkey	1997	PM	476	3.99	-	-	Gargili et al. 1999
Turkey	2006	PM	-	-	68	41.21	Gul A. and Aydin A., 2008
Turkey	2005-2007	ELISA	213	31.4	-	-	Acici et al. 2017
Turkey	NS	PM	1763	4.42	-	-	Kara et al. 2009
Jordan	1999	PM	443	0	-	-	Maraqa et al. 2005

NS: not specified; N: animals tested; F: number of farms; FE: fecal examination under microscope; PM: post mortem examination after slaughtering

Several studies have been conducted in Africa and Asia as concern the prevalence of *Fasciola* in sheep but limited studies have been done in goats (Table 1). In northern Africa, the highest (40%) (Morsy et al., 2005) and the lowest (3.25%) (El-Shazly et al., 2005) prevalence of fasciolosis in sheep was reported in Egypt. Egypt is one of the fasciolosis endemic areas in the world (Amer er al., 2016). The disease burden is high in several species of livestock as well as humans in Egypt (Mekky et al., 2015).

Both species of *Fasciola* are present in sheep in Egypt and the occurrence of the hybrid form has been reported (Amer er al., 2016) and the estimated annual overall costs for fascioliasis in cattle were estimated to be 221.2 USD/cow due to the significant reduction in body weight, reduction in milk production, and the treatment costs for fascioliasis (El-Tahawy et al., 2017). In Turkey, the prevalence of *Fasciola* spp. was found high both in sheep (31,4%) (Acici et al., 2017) and goats (41,21%) (Gul and Aydin, 2008).

The prevalence seems to be higher in southern Europe than in northern Africa and western Asia. The prevalence varies in Europe, being 47.3% in Greece (Kantzoura et al., 2011), 57.4 % in Spain (Perez-Creo et al., 2016), and 95% in Italy (Bosco et al., 2015). In 2014, *F. hepatica* outbreak was reported in Italy and during the four month of observation period, the morbility due to fasciolosis in farms ranged from 3 to 67% and the mortality from 3 to 50% respectively (Bosco et al., 2015).

F. hepatica in southern Europe occupies a climate range, which is mostly warm and dry. Therefore, parasite development and snail reproduction are less constrained by low temperature but they are mostly constrained by lack of moisture resulting in breaks in the life-cycle of the parasite. The actual risk of infection is influenced by the number and distribution of animals, the presence of infected snails and grazing management which allow animals to access herbage or water containing metacercariae (Tum et al., 2004).

The studies carried out in the Mediterranean basin indicated a wide range of prevalence for sheep and goats fasciolosis. These differences are probably due to agro-ecological and climatic differences between the localities, although differences in the management systems may also have resulted in such variation (Abunna et al., 2010). Farms that use private and permanent pastures or wet pastures with mud appeared to be significant risk factors for the infestations (Kantzoura et al., 2011). A geospatial model in Greece suggested that the risk factors of *F. hepatica* were not determined only by herd characteristics, farmer status, farm and pasture management but also by environmental factors (Kantzoura et al., 2011).

The highly infected types of animals in Mediterranean basin are the sheep followed by goats. This observation may be linked to the grazing habits of the two animal species: goats graze on leaves and branches on bushes and trees but sheep graze on plants on the ground where metacercaria are mostly found. So, the possibility of infection with metacercaria is higher in sheep than in goats. Fasciolosis in goats is sporadic in southern Europe whereas in northern Africa and western Asia the highest prevalence (68.4%) is found in Tunisia (Hammami et al. 2007).

Furthermore, *Fasciola* spp infection are well known in small ruminants farms in the Mediterranean basin as the cause of huge financial losses for butchers, farmers and consumers as a result of liver condemnation, poor quality carcass, reduction in growth rate and lower productivity (Mehmood et al., 2017).

***D. dendriticum* infection in small ruminants in the Mediterranean basin**

Dicrocoeliosis is mainly caused by *D. dentriticum* affecting the liver of domestic and wild ruminants, and can lead to weight loss and reduced milk production. This disease is common among grazing ruminants. *D. dentriticum* requires a snail and ants to complete the life cycle and are found in dry lowland or mountain pastures, which provide adequate conditions for the survival and development of terrestrial snails and ants (Otranto and Traversa, 2003). Long-term infection causes progressive hepatic cirrhosis, shortens the reproductive life of sheep and decreases lactation.

Table 2. Prevalence (%) of *Dicrocoelium dendriticum* in small ruminants in the Mediterranean basin.

Country	Year of study	Diagnostic method	Dicrocoelium dendriticum				References
			Sheep		Goats		
			N	%	N	%	
France	1997	FE	-	-	26	15.38	Silvestre et al. 2000
Italy	2004-2005	FLOTAC	121(F)	66.94	-	-	Musella et al. 2011
Italy	2001	FE	NS	6.7	-	-	Sanchez-Andrade et al. 2003
Italy	2001	ELISA	738	86.2	-	-	Sanchez-Andrade et al. 2003
Italy	1999-2000	FE	412	52.3	-	-	Cringoli et al. 2002
Greece	1999	PM	4273	3.1	1292	0.9	Theodoropoulos et al. 2002
Greece	NS	PM	254	16.54	-	-	Katsoulos et al. 2011
Greece	NS	FE	152	20-70	-	-	Mavrogianni et al. 2014
Greece	2006-2007	FE	557	0.2	-	-	Kantzoura et al. 2012
Egypt	NS	FE	50	5	-	-	Haridy et al. 2006
Turkey	1997	PM	476	23.55	-	-	Gargili et al. 1999
Turkey	2006	PM	-	-	70	42.42	Gul A. and Aydin A., 2008
Turkey	2008	PM	1763	3.85	-	-	Kara et al. 2009
Jordan	1999	PM	443	0	-	-	Maraqa et al. 2005

NS: not specified; N: animals tested; F: number of farms; FE: fecal examination under microscope; PE: post mortem examination after slaughtering.

Several studies have been conducted showing the prevalence and geographic distribution of *D. dentriticum* in sheep in southern Europe (Musella et al., 2011; Mavrogianni et al., 2014). The prevalence in sheep was reported to range from 6.7to 86.2% in Italy (Sanchez-Andrade et al., 2003) and from 0.2 to70% in Greece (Kantzoura et al., 2012; Mavrogianni et al., 2014) depending on the examination method used (Table 2). The prevalence of *D. dentriticum* in sheep in Egypt was found to be 5% (Haridy et al., 2006) while in Turkey, the prevalence is reported to range between 3,85- 23,55% in sheep (Gargili et al., 1999; Kara et al., 2009). Data on the prevalence of *D. dentriticum* in goats in southern Europe, northern Africa and western Asia is limited. The prevalence in Turkey is reported to be 42,42% in goats (Gul and Aydin, 2008).

The epidemiology of dicrocoeliosis is also affected by the host species as sheep are more at risk than goats (Theodoropoulos et al., 2002). In Italy, the prevalence of *D. dentriticum* seems to be high in comparison with other countries. Cringoli et al. (2002) found that the rick factors associated with the infection of *D. dentriticum* were the presence of streams/brooks in pastures in ovine farms in Italy. Land use variables such as wood, rocks and arable sparse trees explained the spatial distribution of *D. dentriticum* in Italy (Musella et al., 2011). In southern Europe the landscape is similar especially the presence of seasonal streams and brooks whose banks are generally irregular in shape and covered with tangled bush and undergrowth that provide a hospitable environment for the *D. dentriticum* esogenous stages (Cringoli et al., 2002).

The occurrence of *D. dentriticum* is related to dry and calcareous or alkaline soils, which represent favorable biotopes for their intermediate hosts (Magna-Gonzalez et al., 2001). Diaz et al. (2007) showed that mountainous pastures have a higher risk of infection by *D. dentriticum* since the intermediate hosts of this parasite may find suitable habitats there.

C. daubneyi infection in small ruminants in Mediterranean basin

Paramphistomosis is a parasitic infection of the domestic and wild ruminants caused by trematodes belonging to the family of Paramphistomidae. When immature, the flukes live in the small intestine and abomasum, from where they move to the rumen and become adults (Rojo-Vazquez et., 2012). The success of much of the basic research ultimately hinges on the generation of large scale 'omics' datasets for paramphostomes in general, but particularly for *C. daubneyi* (Huson et al., 2017). *C. daubneyi* requires a snail of the families Planorbidae, Bulinidae and Lymnaeidae to complete the life cycle (Rojo-Vazquez et., 2012).

Clinical *C. daubneyi* infection is still relatively rare in Europe, and is chiefly associated with the feeding activity of excessive numbers of immature stages attached to the mucosal wall of the intestine (Fuertes et al., 2015). *C. daubneyi* is the dominant rumen fluke species in Europe with significant clinical importance in ruminants (Huson et al., 2017). A few work on the epidemiology of *C. daubneyi* in sheep and goats has been done in the Mediterranean area.

Data have been documented in four European and three African countries (Table 3). The prevalence of *C. daubneyi* in sheep in southern Europe and northern Africa was found to be 2.8-16.20% and 2.8-11.8% respectively. Two studies conducted in cattle revealed a prevalence of rumen flukes of 1.2-12.1% in Algeria (Titi et al., 2010) and 36% in Spain (Diaz et al., 2007).

Only one study conducted in goats in France showed a prevalence of 7.69% at farm level (Silvestre et al., 2000). A study conducted in cattle in Algeria found two different species of rumen flukes, *C. daubneyi* and *C. microbothrium* (Titi et al., 2014).

The infection level of sheep and goats with *C. daubneyi* may be underestimated as eggs were not found during coproscopy in infected sheep (Devos et al., 2013), and the seasonality of rumen fluke infestation rates peaked during the winter season (Naranjo-Lucena et al., 2018). The environmental factors such as soil drainage, land cover and habitat were found of major importance for *C. daubneyi* infection in the UK (Naranjo-Lucena et al., 2018). The relevance of these variables is not surprising as soil drainage will affect the moisture level and consequently the development of snail intermediate host (Naranjo-Lucena et al., 2018).

The life cycle of *C. daubneyi* shares some features with that of *F. hepatica*, involving the same intermediate and definitive hosts (Naranjo-Lucena et al., 2018). Thus, a positive correlation between the prevalence of *F. hepatica* and *C. daubneyi* (Musella et al., 2011) was observed, as well as a similarity in some potential risk factors (vegetation and rainfall variables) for both parasites (Naranjo-Lucena et al., 2018). There is also a possible interaction between infection with the two parasites (*F. hepatica* and *C. daubneyi*) in sheep in Ireland, where climatological and environmental conditions are particularly favorable for the development of both flukes and their intermediate hosts. Rumen fluke models showed that average number and timing of treatments against *F. hepatica* are positive predictors for *C. daubneyi* infection (Jones et al., 2017).

Table 3. Prevalence (%) of *Calicophoron daubreyi* in small ruminants in the Mediterranean.

Country	Year of study	Diagnostic method	*Calicophoron daubreyi*				References
			Sheep		Goats		
			N	%	N	%	
France	1997	FE	-	-	16(F)	12.5	Silvestre et al. 2000
Italy	2004-2005	FLOTAC	121(F)	14.05	-	-	Musella et al. 2011
Italy	2000-2001	FE	197(F)	16.20	-	-	Cringoli et al. 2004
Italy	2011-2014	FLOTAC	381	13.9%	-	-	Sanna et al. 2016
Tunisia	2004-2005	FE	36	2.8	-	-	Akkari et al. 2012
Egypt	NS	NS	NS	11.8	-	-	Mazyad et al. 2002
Egypt	NS	FE	50	4	-	-	Haridy et al. 2006
Turkey	2008	PM	1763	0	-	-	Kara et al. 2009

NS: not specified; N: animals tested; FE: fecal examination under microscope; PM: post mortem examination after slaughtering.

Furthermore, some studies showed that the infection rates for rumen fluke were significantly and consistently lower in sheep than in cattle (Toolan et al., 2015; Sanna et al., 2016), with the odds of being positive for rumen fluke eggs about 3 times higher in bovine than in ovine submissions (Naranjo-Lucena et al., 2018).

Rumen fluke should now be considered as an emerging parasitic infection of ruminant livestock in Europe (Huson et al., 2017), with a significant increase in the number of free rediae (Mage et al., 2002) and a high prevalence of infection observed in small ruminants in many countries in the Mediterranean basin.

Conclusion

The most prevalent trematode infections in cattle in Europe and Africa are frequently caused by flukes belonging to the genera *Fasciola*, *Dicrocoelium* and *Paramphistomum*. Although many studies regarding ruminant infection by *F. hepatica* have been conducted in Europe, only a few studies concerning the infection by *D. dendriticum* and *C. daubneyi* have been developed in Europe, Asia and Africa in small ruminants.

The high prevalence of *Fasciola* spp. in small ruminants found in several countries of the Mediterranean basin demonstrates that control measurers currently used are not sufficient to control the infection. Alterations in the epidemiology of *F. hepatica* resulting from climate change will influence the profitability of sheep farmers in southern Europe and probably also in other regions in the Mediterranean area. *C. daubneyi* should be considered as an emerging parasitic infection of ruminant livestock in the Mediterranean area, and its life cycle shares features with that of *F. hepatica* involving the same intermediate and definitive hosts. *D. dentriticum* is present in many European countries but further research should be done in Africa. The best approach to restrict the occurrence of diseases caused by flukes is to implement forms of integrated parasite control based on proper husbandry practices and strategic treatments.

Competing interests

Nothing to declare

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