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Final destination: The Mediterranean Sea, a vulnerable sea. The long journey of *Giardia duodenalis* cysts

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ABSTRACT

The Mediterranean Sea is considered a "litmus paper" of pollution risks for any parameter, including faecal contamination. *Giardia duodenalis* is one of the most important protozoan parasites responsible for diarrhoea in a wide range of hosts, including humans, domestic and wild animals, worldwide. The degree of contamination related to the protozoan's resistant forms on land, and the consequent transport through rivers from point sources to the sea are important aspects to better understand the processes involved in the microbiological pollution of aquatic ecosystems. However, land-sea transfer routes and the complex transmission patterns often remain neglected. This contribution deals with the contamination by *G. duodenalis* of the Mediterranean Sea through its inhabitants (shellfish, marine mammals, fishes), and provides data on the origin of such contamination on land from humans and animals to soil, fresh produce and waters; this scenario allows to understand the long journey of the protozoan following the drainage basins (i.e., natural watersheds) from the mainland towards the final destination. The Mediterranean Sea contamination is also explained in the light of the *Giardia* survival in water and the effects of climatic change with the related consequences. Addressing faecal contamination threats in the Mediterranean Sea is a difficult task, but a number of mitigation measures need to be implemented and/or in some countries even applied. Effective management must become a priority in the agenda of policy makers of all Mediterranean Countries for the implementation of successful measures and can only be applied in the perspective of the One Health approach.

1. Introduction

1.1. The Mediterranean Basin

The Mediterranean Basin has a unique feature. It includes parts of Europe and Africa and extends into Asia, covering the western and southern areas of the peninsula of Turkey, Israel, and Lebanon. With the exception of the 200 m-wide Suez Canal and the 14 km-wide Strait of Gibraltar, it is entirely enclosed. The Mediterranean Basin's coast extends for a total length of 46.000 km and it is surrounded by 22 countries, including Malta and Cyprus islands. It is also dotted by a myriad of islands (Fig. 1). The coastline is irregular and deeply indented with a high variety of geographic characteristics: high mountains and rocky shores, thick scrub and semi-arid steppes, coastal wetlands and sandy beaches.

Climatically, the Mediterranean Basin is characterised by warm to hot dry summers and mild, fairly wet winters (<250–2500 mm); however, important variations depend on geographical gradients (García--Herrera et al., 2018).

The hydrographic basin of the Mediterranean Sea is particularly heterogeneous and extends beyond the Mediterranean region. Indeed, some European and African countries are located in the drainage basin of the Mediterranean with or without a coastline on the Mediterranean Sea (Lionello, 2012) (Fig. 1). The catchment area accounts for about 4,

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135×10^3 km^2. The watershed is characterized by the presence of the huge Nile and over 200 rivers of various sizes flowing along its coast. The potential annual water load estimation for the rivers is about 550 km^3 (Poulos et al., 2011).

The population of the Mediterranean Basin is approximately 480 million inhabitants, mostly concentrated near the coasts (European Environment Agency, 2020).

The livestock population consists of approximately 26,3 million cattle, 126,2 million sheep and 33,9 million goats (deRancourt and Mottet, 2008) besides an unknown number of camels, equines, and pigs. Dog population accounts for over 1,3 million (Bedford, 2022) also the cat population is very numerous, but never quantified.

More than 85% of the total agricultural output in the Mediterranean is made up of cereals, vegetables, and citrus fruits. A sizable portion of agricultural land is also used to grow other products like grapes and olives (Leff et al., 2004).

Agriculture requires fertilisers (including animal manure) and irrigation water, putting excessive pressure on the environment. Up to 80% of the available water is used for irrigation (Thivet and Blinda, 2007).

The Mediterranean region is a leading tourist destination in the world. Tourism is primarily based on summer holidays and it is mainly concentrated in the coastal areas which welcome 30% of the international tourist arrivals.

Due to the intense anthropogenic pressures and atmospheric precipitation, inland basins and rivers accumulate the products derived from human activities (wastewater, animal manure, human faecal waste, etc.) which are transported downstream up to the river mouths and then to the sea (PERSEUS-UNEP/MAP, 2015).

The ecological pressure caused by intense anthropic activities means that all marine environments can be considered reliable indicators (as mentioned above, a sort of "litmus test") of environmental fecal pollution, including coliform contamination (Gunda et al., 2017), as evidenced by the presence of viral, bacterial, and protozoan pathogens in marine waters (UNEP/MAP, 2010).

1.2. From land to sea

Wastewater outfalls and runoff from rural, suburban and urban landscapes can carry a variety of resistant forms of parasites (e.g. protozoan oo/cysts, helminth eggs) into waters, some used for agriculture (often untreated) - thus contaminating soil and produce - some for recreational areas, and others even for drinking water (Noda et al., 2009). Parasitic pathogens can reach estuaries and contaminate the seawater where they are filtered and concentrated by shellfish (most of them edible) or are swallowed by a range of marine animal hosts (Fayer et al., 2004; Giangaspero et al., 2009; Aksoy et al., 2014; Ligda et al., 2019) (Fig. 2).

The degree of contamination by protozoan-resistant forms and the

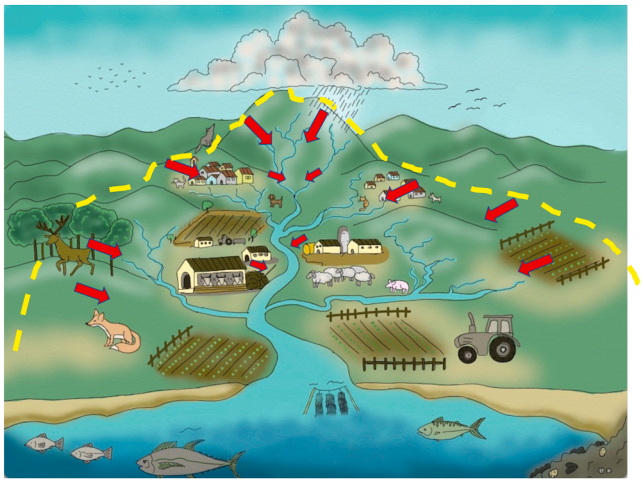

Fig. 2. Boundaries of a watershed and the potential contamination sources. Water is channelled into soils, groundwater, creeks, and streams, making its way to larger rivers and eventually the sea.

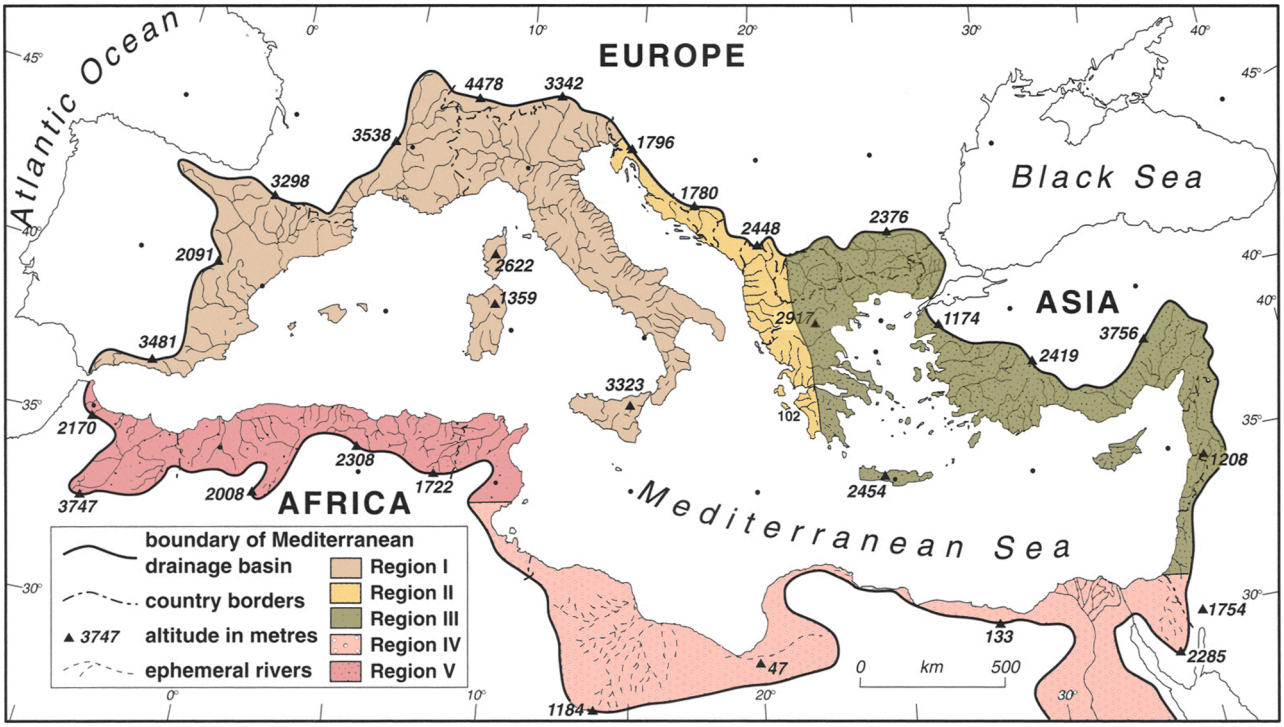

Fig. 1. Rivers and their drainage basins along the Mediterranean Basin including its division into five physical geographical regions (I–V). The Nile basin is only partially shown (adapted from Poulos and Collins, 2002).

resultant transport through rivers from the point sources to the sea are, therefore, important to better understand the processes underlying the contamination of aquatic ecosystems with pathogens. However, land-sea transfer routes and the complex transmission patterns involved, often remain neglected.

1.3. *Giardia duodenalis* as a model of intestinal parasite contamination

Giardia duodenalis (syn. *Giardia lamblia* and *Giardia intestinalis*) is one of the most important protozoan parasites responsible for diarrhoea in a wide range of hosts, including humans and domestic or wild animals (Robertson et al., 2020). The infection occurs through the ingestion of cysts via contaminated food or drink, or direct contact with infected animals or persons. There are currently nine recognized species of *Giardia: Giardia agilis, Giardia ardeae, Giardia psittaci, Giardia varani, Giardia muris, Giardia microti, Giardia peramelis, G. cricetidarum* and *Giardia duodenalis* (Ryan et al., 2021). However, only the latter infects humans and a broad range of mammals. *G. duodenalis* is a species complex made up of eight assemblages (A–H). Assemblages A and B have a broad host range (including humans and other mammals) and are primarily responsible for human giardiasis. In cats and dogs, assemblages A, B, C, D, and F are commonly reported with a different range of host specificity, whereas livestock (cattle, buffaloes, sheep, goats, pigs) and wild ruminants (ungulates) are predominantly infected with *G. duodenalis* assemblage E. Assemblages C, D, E, and F have been sporadically reported in humans. Rodents are infected by assemblage G and pinnipeds by assemblage H (Cacciò et al., 2018). Currently, based on molecular analysis and host association, some authors propose to name assemblages as species (Wielinga et al., 2023).

Asymptomatic infections are more common, but, in immunocompromised subjects, giardiasis can cause severe diarrhoea associated with weight loss, abdominal pain and rarely mortality (Horton et al., 2019).

Although each infected host often excretes large numbers of cysts, relatively few are required (between 10 and 100 cysts) to initiate an infection (Roxstrom-Lindquist et al., 2006). Furthermore, they are small and resistant to the most commonly used disinfectants, and can survive in moist environments for a long period, even when exposed to a wide range of temperatures and salinities (Wang et al., 2023). With regard to the viability, as an example, it has been shown that *Giardia* cysts can remain viable in water and be infective to mice for up to one month at temperatures as low as 4 °C (Faubert et al., 1986). These peculiar characteristics can contribute to a rapid and widespread environmental dispersal through soil and water.

Given the detection of the resistance forms (cysts), tap water, surface waters, estuarine and coastal waters, soil, vegetables, fruits and marine organisms are considered reliable indicators of widespread faecal contamination from land to sea by *G. duodenalis* (Hilles et al., 2014). However, such a large spread – which also involves coliforms (Liang et al., 2023) - has an impact on human health that is likely underestimated.

From this perspective, *Giardia* cysts represent a valid model for understanding the ``vulnerable nature'' of the Mediterranean Sea and the effects of such contamination, which are capable of determining a vicious circle between animals, humans and the environment.

The present contribution deals with the contamination by *G. duodenalis* of the Mediterranean Sea through its inhabitants, and provides data on the origin of such contamination on land, from humans and animals to soil, fresh produce and waters, in order to understand the long protozoan journey following the natural water discharges from the mainland towards the final destination.

2. Literature search strategy and selection process

For this review, we screened articles published (indexed and printed) between January 2011 and December 2022 from 25 countries. These include the European, Asian and African countries surrounding the Mediterranean Sea, those territories, or part thereof, included within the drainage basin of the Mediterranean Sea, two island nations, and a British Overseas Territory. In clockwise order, they are Gibraltar, Spain, France, Italy, Malta, Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, Albania, Kosovo, North Macedonia, Bulgaria, Greece, Turkey, Syria, Cyprus, Lebanon, Israel, Palestine (Gaza Strip), Egypt, Libya, Tunisia, Algeria and Morocco[2].

Therefore, for each country, only contributions investigating areas/regions included within the drainage basin of the Mediterranean Sea (as shown in Fig. 1) were taken into consideration. The publications cited in this narrative Review were identified through comprehensive searches of PubMed, Web of Science, Google Scholar, Science Direct, Scopus, and scientific reports were explored to retrieve relevant publications.

For references cited in the whole contribution, searches were conducted using the terms "Mediterranean Basin" AND "geography" OR "climate" OR "hydrography" OR "watershed" OR "population" OR "agriculture" OR "animal population" OR "economy"; "Mediterranean Basin" AND "climatic changes"; "Mediterranean Basin" AND "pollution"; "*Giardia*" AND "taxonomy" OR "genotype"; "*Giardia*" AND "life-cycle" OR "transmission"; "giardi*" AND "symptoms" OR "clinics"; "*Giardia*/giardi*" AND "epidemiology" OR "prevalence" AND "animal" OR "human" OR "soil" OR "fresh produce" OR "waters" OR" shellfish" OR "fish" OR "marine water" AND "Gibraltar" OR "Spain" OR "France" OR "Italy" OR "Malta" OR "Slovenia" OR "Croatia" OR "Bosnia and Herzegovina" OR "Montenegro" OR "Albania" OR "Kosovo" OR "North Macedonia" OR "Bulgaria" OR "Greece" OR "Turkey" OR "Syria" OR "Cyprus" OR "Lebanon" OR "Palestine (Gaza Strip)" OR "Israel" OR "Palestine" OR "Egypt" OR "Libya" OR "Tunisia" OR "Algeria" OR "Morocco"; "*Giardia*" AND "viability" OR "resistance"; "giardi*" AND "sanitation" OR "hygiene"; "giardia*" AND "prevention" OR "control".

We screened the titles and abstracts with consistent content and context and the full articles were read to verify their relevance to the present topics in this Review. A total of 237 peer-reviewed research articles were identified.

3. *Giardia duodenalis* in sea water and marine animals: the Mediterranean Sea hotspot

According to Table 1, the presence of *G. duodenalis* has been documented in sea water and some species of marine animals inhabiting the Mediterranean Sea. As to sea water, in the eastern Mediterranean, *G. duodenalis* cysts have been detected by microscopy in 1.9% of 52 marine water samples and in 1/6 sites along the coastline of Gaza (Hilles et al., 2014).

Edible shellfish were the most investigated group of organisms. Reported prevalence rates showed great variability, ranging from a low prevalence (2%) in Tunisia's coast (Ghozzi et al., 2017) to a high prevalence (23.3%) as single or associated contamination with *Cryptosporidium* (Giangaspero et al., 2014). Other investigated groups of marine animals include mammals and fishes with prevalences of 12–16% in free-living whales (Hermosilla et al., 2018) and dolphins (Marangi et al. 2022) and 3.3% in farmed and free-living fishes (Ghoneim et al., 2012). Molecular techniques were largely used in marine animals to detect the presence of *Giardia*. All the genotyped isolates were belonging to the zoonotic assemblage A (Ghoneim et al., 2012; Giangaspero et al., 2014; Marangi et al., 2015; Ghozzi et al., 2017; Tedde et al., 2019; Marangi et al., 2022).

The fact that the Mediterranean Sea can be considered a "litmus test"

[2] Andorra, Principality of Monaco, Switzerland, San Marino, the Vatican and Serbia, which play a negligible role in the contamination, were not included. A peculiar case is represented by the transboundary river Nile: although its watershed represents almost 70% of the Mediterranean drainage basin, its fresh water input is very low (Poulos, 2011). Therefore, only the drainage basin of Egypt has been included in the study (Figure 1).

Table 1
Giardia duodenalis cysts/DNA (Number of positive samples/number of examined samples (%)) in sea water and marine animal species (mammals, mollusks, fishes) of the Mediterranean Sea. Results are presented in chronological order (2011–2022) according to the country.

Country	Animal group/ water	Animal species	Method/s used	Positive/total (%)	Assemblages	Refs.
Spain	Whales	*Physeter macrocephalus*	Microscopy, ELISA	4/25 (16)[a]	–	Hermosilla et al., 2018
Italy	Mussels	*Mytilus galloprovincialis*	PCR	14/60 (23.3)[b]	A	Giangaspero et al., 2014
	Mussels	*Mytilus galloprovincialis* *Mytilus edulis*	PCR	19/135 (14)[c]	A	Tedde et al., 2019
	Dolphins[d]	*Stenella coeruleoalba*	PCR	2/11 (18.2)[a]	A	Marangi et al., 2022
Palestine (Gaza Strip)	Sea water	–	Microscopy	1/52 (1.9)	–	Hilles et al., 2014
Egypt	Fishes	*Tilapia nilotica* *Mugil cephalus*	ELISA, PCR	3/92 (3.3)[a]	A	Ghoneim et al., 2012
Tunisia	Clams, oysters, mussels	*Ruditapes decussatus* *Pinctada radiata* *Mytilus galloprovincialis*	PCR	2/87 (2)[e]	A	Ghozzi et al., 2017

"–" Not applicable, not investigated or not reported. [a]Faecal samples; [b]Pools of 15 mussels each; [c]Pools of 12 mussels each; [d]Free-living; [e]Pools of 9–18 specimens each; ELISA: Enzyme linked immunosorbent assay; PCR: polymerase chain reaction.

is demonstrated by the contamination registered in organisms inhabiting the sea. Indeed, resistant *Giardia* cysts can be accumulated in bivalves (Giangaspero et al., 2014; Marangi et al., 2015; Ghozzi et al., 2017; Tedde et al., 2019) or be swallowed by other organisms. As far as bivalves are concerned, it is important to underline that, due to their highly filtering behaviour, they can be considered as bio-sentinels for seawater contamination with chemical pollutants and also for protozoan parasites, such as *G. duodenalis* (WHO, 2010).

As to fish, *Giardia* cysts can be present in both freshwater and marine fish species of commercial interest such as mango fish (*Tilapia nilotica)* and flathead grey mullet (*Mugil cephalus*). Although cysts accumulate in the intestine of fish, which is usually not consumed and discarded, there is anyway a risk of contamination of edible parts and risk of infection for workers handling the intestinal tract during evisceration and other procedures (Ghoneim et al., 2012).

Detection in sea organisms of *G. duodenalis* assemblage A (the most diffused and zoonotic assemblage) may confirm that sea matches what has been evidenced on land, i.e. that this assemblage is the most widespread. However, it is not excluded that other assemblages may be detected in Mediterranean marine animals. In this view, the lack of epidemiological research conducted to date in these animal species is certainly insufficient to get conclusions. Moreover, the potential role of the consumption of shellfish and some species of fish as a source of giardiasis should be investigated more in depth.

4. The long journey of *Giardia duodenalis*

4.1. Prevalence in humans

As illustrated in Table 2, the prevalence of *G. duodenalis* in the Mediterranean human population has been documented in 20 countries. We identified a large number of studies ($n = 42$) in Egypt. Overall, over 450 thousand faecal samples were examined, including both symptomatic and asymptomatic adults and children, by different techniques. Reported prevalence values show great variations, ranging from a prevalence as low as 0.07% in food workers in Croatia (Plutzer et al., 2018) to 56.9% in a group of symptomatic adults and children, in Egypt (Yu et al., 2019).

G. duodenalis assemblages in humans have been investigated to a lesser extent, since data were available from 10 countries. As expected, A, B, and mixed A/B assemblages were identified, with some studies showing a great variability among sub-assemblages (see Table 2 for references). Surprisingly, assemblage C (Soliman et al., 2011) and E (Helmy et al., 2014; Abdel-Moein et al., 2016), commonly considered as non-zoonotic and host specific, were detected in humans, as reported in some studies from Egypt.

To the best of our knowledge, human giardiasis is not a compulsory notifiable disease in any Mediterranean countries, thus, the cases along the Mediterranean Basin are difficult to assess. The prevalence data reported in Table 2 show a patchy distribution and can only give an idea of the infection presence in this geographic area. Several studies detected the infection in paediatric populations, patients with various pathological features, and symptomatic subjects, with diarrhoea being the most common clinical manifestation. Some Italian studies also reported the infection in groups of immigrants seeking medical assistance and, sometimes, some of them were infected but asymptomatic (Gualdieri et al., 2011; Masucci et al., 2011; Manganelli et al., 2012; Silvestri et al., 2013; Cobo et al., 2016; Bartolini et al., 2017). Because of the poor socio-economic conditions and difficulty in accessing medical care, these individuals may remain largely untreated.

4.2. Prevalence in animals

Data on contamination by *G. duodenalis* in animals are reported in Table 3 and available from 14 Mediterranean countries, i.e., Spain, France, Italy, Slovenia, Croatia, Bosnia Herzegovina, Albania, Greece, Turkey, Cyprus, Israel, Egypt, Algeria, Morocco.

Overall, over 26,500 faecal samples were examined, in both symptomatic and asymptomatic animals, by different techniques.

G. duodenalis have been reported to infect numerous animal species including dogs, cats, sheep, cattle, swine, horses, buffaloes, rats, wild animals (chamois, porcupines, badgers, wolves, chinchillas, reptiles, foxes), several species of zoo animals (non-human primates, birds, lemurs) (See Table 3 for references).

With the exception of Slovenia, Algeria, and Egypt, dogs were the most tested companion animal species in all the examined countries, with prevalence up to 57% in kenneled dogs (Simonato et al., 2015). The prevalence in livestock was high in all the investigated countries, reaching 89.2% in lambs in Spain (Gomez-Munoz et al., 2012). Among zoo and wild animals, it is worth noting the high prevalence recorded in captive non-human primates (Berrilli et al., 2011) and, unexpectedly, in porcupines (48.8%) (Coppola et al., 2020) and badgers (50%) in Italy (Maestrini et al., 2022).

Seven *G. duodenalis* assemblages were reported in these studies, according to the investigated species (see Table 3 for references). Despite species-specific assemblages having been detected, zoonotic assemblage A and mostly human related assemblage B are commonly found in most of all animal species, including wild animals, followed by C and D (in companion animals), E (in livestock), F (in cats) and G (in rats).

As shown, most of the studies carried out on the presence of *Giardia* in animals deals with companion animals and in particular dogs, cats and, to a lesser extent, other pets (Table 3). This aspect is related to the need to investigate the zoonotic role of *Giardia* due to the close contact of humans with these animal species. However, although not many contributions have been published on livestock in the examined countries, their role in contributing to the environmental contamination cannot be underestimated. Livestock infected with *Giardia* shed large numbers of cysts, and they are more able to contaminate food and/or

Table 2
Giardia duodenalis cysts/DNA (Number of positive samples/number of examined samples (%)) in humans from Mediterranean draining Basin. Results are presented in chronological order (2011–2022) according to the country.

Country	Human group	Method/s used	Positive/total (%)	Assemblage/sub-assemblage*	Refs.
Spain	Children, adults	Microscopy	321/8,313 (3.9)	–	González-Moreno et al., 2011
	Children	ELISA, ICT, Microscopy, PCR	10/325 (3.1)	AII, B	Cardona et al., 2011
	Hosted Saharawi children	Microscopy	78/270 (29)	–	Soriano et al., 2011
	Immigrants	Microscopy	111/2,426 (4.6)	–	Cobo et al., 2016
	Traveler symptomatic children	–	61/606 (10.1)	–	Soriano-Arandes et al., 2016
	Patients	Microscopy, ICT, PCR	106/209	AII, AIII, BIII, BIV	Azcona-Gutiérrez et al., 2017
	Adult HIV patients with enteritis	Microscopy, ICT	8/73 (11)	–	Fernández-Huerta et al., 2019
	Symptomatic adult patients	Microscopy, ICT, EIA Ig A,	69/269 (26.5)	–	Trelis et al., 2019
	Healthy adult individuals	ELISA Ig A	1/82 (1.2)		
	Outpatients	PCR	125/125	A, B	Wang et al., 2019
	Children	Microscopy, ICT, PCR	190	–	Saura-Carretero et al., 2021
	Obese patients	PCR	9/104 (8.7)	–	Caudet et al., 2022a
	Obese patients	PCR	9/56 (16.1)	–	Caudet et al., 2022b
	Symptomatic traveler adults	ICT	113/651 (17.4)	–	España-Cueto et al., 2022
	Children	Microscopy	8/500 (1.6)		Mormeneo Bayo et al., 2022
		PCR	35/500 (7)		
France	Symptomatic children	PCR	2/529 (0.37)	–	Fenollar et al., 2016
	Healthy children		5/3119 (0.16		
	HIV-infected patients	PCR	3/39 (7.7)	–	Hamad et al., 2018
	Referred patients	Microscopy, PCR	11/488 (2.3)	–	Menu et al., 2019
Italy	Immigrants	Microscopy	23/514 (4.5)	–	Gualdieri et al., 2011
	Italian, immigrant patients	Microscopy	69/5,351 (1.3)	–	Masucci et al., 2011
	Adult MSM	IFA	11/66 (16.6)	–	Di Benedetto et al., 2012
	Immigrant children	Microscopy, IFA	13/247 (5.3)	–	Manganelli et al., 2012
	Adult, children, Italian, immigrant patients	Microscopy	99/5,323 (1.8)	–	Silvestri et al., 2013
	Symptomatic patients	Microscopy, ICT, IFA	168/8,886 (1.89)	–	Calderaro et al., 2014
	Symptomatic children	–	1/64 (3.3)	–	Pensabene et al., 2015
	Italian, immigrant patients	Microscopy, IFA	69/7,341 (0.94)	–	Bartolini et al., 2017
	Symptomatic children	Microscopy, ICT, IFA, PCR	11/1,716 (0.6)	–	Calderaro et al., 2018
	Immunocompromised patients	Endoscopy	3/58 (5.2)	–	Varricchi et al., 2018
	Symptomatic, asymptomatic patients	Microscopy, ICT, PCR	228/854 (26.7)	AI, AII, AIII, B	Resi et al., 2021
Slovenia	Symptomatic patients	IFA, PCR	85	A, B	Soba et al., 2015
	Symptomatic patients	Microscopy, IFA	237/24,782 (0.96)	–	Plutzer et al., 2018
Croatia	Food workers	Microscopy	164/245,321 (0.07)	–	Plutzer et al., 2018
	Symptomatic patients	Microscopy	41/17,183 (0.24)	–	
Bosnia and Herzegovina	Symptomatic patients	Microscopy, IFA	1/11 (9.09)	–	Plutzer et al., 2018
Albania	Children	Microscopy	35/321 (10.9)	–	Sejdini et al., 2011
Bulgaria	Symptomatic children	ICT	5/115 (4.3)	–	Mladenova et al., 2015
	General population	Microscopy	3,768/1,391,113 (0.27)	–	Harizanov et al., 2020
Kosovo	Symptomatic children	Microscopy	79/530 (14.9)	–	Korzeniewski et al., 2020
			6/310 (1.9)		
Greece	Children, adults	Microscopy, IFA, PCR	11/876 (1.3)	AII	Kostopoulou et al., 2020
Turkey	Food workers	Microscopy	13/500 (2.6)	–	Bayramoğlu et al., 2013
		ICT	8/500 (1.6)		
		IFA	24/500 (4.8		
	Symptomatic patients	Microscopy, PCR	32/39 (82)	AII, AIII, BII, BIII, BIV	Çiçek and Şakru, 2015
	Asymptomatic patients		19/39 (48.7)		
	Patients	Microscopy, PCR	40	AI, AII, AIII, B	Ertuğ et al., 2016
	Immunocompromised, adult patients	Microscopy	7/26 (26.9)	–	Uysal et al., 2016
	Symptomatic patients	Microscopy, ELISA, PCR	7/272 (2.6)	–	Koltas et al., 2017
	Symptomatic immunocompromised children	Microscopy	3/62 (4.8)	–	Caner et al., 2020
	Patients	Microscopy	70/18,450 (0.3)	–	Ergüden Gürbüz et al., 2020
	General population	–	2/2233 (0.09)	–	Zorbozan 2022
Syria	Hospitalized patients	Microscopy, PCR	40	A, B	Skhal et al., 2016
	Symptomatic patients	PCR	40	AI, AII, BIII, BIV	Skhal et al., 2017
Lebanon	Tertiary care center patients	Microscopy	394/22,248 (1.8)	–	Araj et al., 2011
	School children	Microscopy, PCR	71/249 (28.5)	A, B	Osman et al., 2016
Israel	Children	Microscopy	5,207/45,978 (11.3)	–	Ben-Shimol et al., 2014
	Symptomatic children	Microscopy	21/142 (14.8)	–	Muhsen et al., 2014
	Symptomatic travelers	PCR	6/203 (3)	–	Gefen-Halevi et al., 2022

(*continued on next page*)

Table 2 (*continued*)

Country	Human group	Method/s used	Positive/total (%)	Assemblage/sub-assemblage*	Refs.
	Asymptomatic non-travelers		20/1359 (1.5)		
	Symptomatic children	PCR	6/140 (4)	–	Sever et al., 2022
Palestine (Gaza Strip)	Symptomatic children, adults	Microscopy	18/319 (5.6)	–	Al-Hindi and Shammala, 2013
	Patients	Microscopy	57/600 (9.5)	–	Mezeid et al., 2014
	Symptomatic, asymptomatic children	Microscopy	40/150 (26.7)	–	Al Laham et al., 2015
	University female students	Microscopy	15/305 (4.9)	–	Al-Hindi et al., 2019
	Healthy controls		2/10 (10)		
Egypt	Symptomatic children	Microscopy, PCR	12/130 (9)	A, B, C	Soliman et al., 2011
	Diarrheic oncologic patients		3/20 (15)		
	Immunosuppressed, immunocompetent, symptomatic children	Microscopy	33/450 (7.3)	–	Abdel-Hafeez et al., 2012
	Pregnant women	Microscopy	2/200 (1)	–	El Deeb et al., 2012
	Outpatients	ICT	89/391 (22.8)	–	Abd El Kader et al., 2012
	Symptomatic children	EIA	146/2,112 (7)	–	El-Mohammady et al., 2012
	Clinical samples	PCR	48	AI, AII, B	Amer et al., 2013
	Symptomatic patients	Microscopy, PCR	122/598 (20.4)	–	Nazeer et al., 2013
	Healthy controls		151/598 (25.2)		
	Symptomatic, healthy children	Microscopy	77/180 (42.7)	–	Eldash et al., 2013
	Symptomatic children	ICT	13/165 (8)	A, AII, B, E	Helmy et al., 2014
		PCR	35/165 (21)		
	Dyspeptic patients	Microscopy, PCR	23/120 (19.2)	A, B	Fouad et al., 2014
	Symptomatic patients	Microscopy, ICT	16/104 (15.4)	–	Banisch et al., 2015
	Children with conjunctivitis	Microscopy	1/50 (2)	–	El Hady et al., 2015
	Diabetic patients, controls	Microscopy	27/200 (13.5)	–	Elnadi et al., 2015
	Children with chronic liver disease	Microscopy	7/50 (14%)	–	El-Shazly et al 2015
	Symptomatic children		9/50 (28%)		
	Symptomatic children	PCR	77/96 (80)	A, AII, B	Fahmy et al., 2015
	Symptomatic patients	Microscopy	6/206 (3)	–	Sabah et al., 2015
	Outpatients	Microscopy, ELISA, ICT	15/90 (16.6)	–	Sadaka et al., 2015
	Children	Microscopy	28/120 (23.3)	–	Shalaby and Shalaby, 2015
	Mentally handicapped individuals	Microscopy	17/200 (8.5)	–	Shehata and Hassanein, 2015
	Symptomatic children	PCR	25/40 (62.5)	E	Abdel-Moein et al., 2016
	Symptomatic patients, healthy controls	Microscopy	32/91 (35.2)	–	Atia et al., 2016
		ICT	34/91 (37.4)		
	Children	Microscopy	62/1,615 (3.8)	–	Bayoumy et al., 2016
	Children	Microscopy	11/300 (5.3)	–	Dyab et al., 2016
	Solid-waste workers	Microscopy	10/346 (2.9)	–	Eassa et al., 2016
	Symptomatic children, adults	Microscopy, PCR	62/400 (15.5)	A, B	El Basha et al., 2016
	Patients (2–58 yo)	Microscopy, ELISA	71/185 (38)	–	Elswaifi et al., 2016
	SLE patients	Microscopy	28/40 (70)	–	Fawzi et al., 2016
	Healthy controls		29/30 (96.7)		
	Anaemic children	Microscopy, PCR	145/650 (22.3)	AI, AII, B	Hussein et al., 2016
	Symptomatic patients	Microscopy, PCR	224/1187 (18.9)	A, B	Ismail et al., 2016
	Symptomatic patients	Microscopy, PCR	80/801 (10)	AII, BIII, BIV	El-Badry et al., 2017
	Symptomatic, asymptomatic children	Microscopy, PCR	65/660 (9.8)	AI, AII, B	Hussein et al., 2017
	Students	Microcopy	41/600 (6.8)	–	Bayoumi et al., 2018
	Children	Microscopy	3/796 (0.4)	–	Elfadaly et al., 2018
	Symptomatic, asymptomatic children	PCR	585 (11.3)	A, AII, B	Naguib et al., 2018b
	Symptomatic children	Microscopy	49/400 (12.25)	–	Geneidy 2019
	Hemodialysis patients	Microscopy	2/120 (1.7)	–	Shehata et al., 2019
	Healthy controls		4/100 (4)		
	Symptomatic children, adults	Microscopy, PCR	181/318 (56.9)	A, B	Yu et al., 2019
	Symptomatic children	Microscopy, PCR	24/100 (24)	AII	Abd El-Latif et al., 2020
	Symptomatic, asymptomatic children	Microscopy, PCR	40/165 (24.2)	A, B	Ahmad et al., 2020
	Children	Microscopy, PCR	57/315 (18.1)	A, B	Elhadad et al., 2022
	Infected children, control	Microcopy, PCR	255/1260 (20.23)	A, B	Ismail et al., 2022
	Symptomatic patients	Microscopy	63/500 (12.6)	–	Omar and Abdelal 2022
Libya	Symptomatic, asymptomatic, children, adults	–	(4.6)	–	Ghenghesh et al., 2016
	Symptomatic children	Microscopy, IFA	133/505 (26.3)	–	Saaed et al., 2019
Tunisia	Food handlers	Microscopy	103/8,502 (1.2)	–	Siala et al., 2011
Algeria	Symptomatic, asymptomatic children	Microscopy, PCR	30/355 (8.4)	A, B	Rebih et al., 2020
	Outpatients, hospitalized patients	Microscopy	97/2,504 (3.9)	–	Belkessa et al., 2021a
	Suspected patients	Microscopy, PCR	80/119 (67)	A, B	Belkessa et al., 2021b
Morocco	School children	Microscopy, PCR	84/673 (12.5)	AII, BIII, BIV	El Fatni et al., 2014

"–" Not applicable, not investigated or not reported. *Single or mixed genotypes; IFA: Immunofluorescence assay; ICT: immunochromatographic test; EIA: Enzyme immuno assay; ELISA: Enzyme linked immunosorbent assay; PCR: polymerase chain reaction.

Table 3
Giardia duodenalis cysts/DNA (Number of positive samples/number of examined samples (%)) in different animal species in the Mediterranean drainage Basin. Results are presented in chronological order (2011–2022) according to the country.

Country	Animal group	Species	Method/s used	Positive/total (%)	Assemblage/sub-assemblage*	Refs.
Spain	Captive non-human primates	Several species	Microscopy, PCR	14/20 (70)**	A, B	Martínez-Díaz et al., 2011
	Livestock	Cattle	ELISA, ICT, PCR	227 (1.4)	E, A, B	Cardona et al., 2011
	Livestock	Lambs	IF, PCR	107/120 (89.2)	A, E	Gomez et al., 2012
	Companion animals	Dogs		64/169 (37.4)	C, D	Ortuño et al., 2014
	Companion animals	Dogs	IF, PCR	127/348 (36.5)	A, B, C, D	Adell-Aledon et al., 2018
	Companion animals	Dogs	Microscopy, IF	93/263 (35.4)	–	Sanchez-Thevenet et al., 2019
	Livestock	Swine	PCR	64/328 (19.5)	A, E	Rivero-Juarez et al., 2020
	Synanthropic animals	Rats	PCR	35/100 (35)	G	Galán-Puchades et al., 2021
	Non-human primates	Several species	PCR	11/51 (21.6)	AII	Köster et al., 2021
	Wild animals	*Rattus* spp.		9/64 (14.1)	G	
	Livestock	Swine	PCR	48/475 (10.7)	E	Dashti et al., 2022
	Companion animals	Dogs	IF	99/365 (27.1)	–	Remesar et al., 2022
	Captive non-human Primates	Several Species	Microscopy, PCR	23/79 (29.1)	AII, B, BIV	Köster et al., 2022
France	Companion animals	Dogs	Microscopy, PCR	29/116 (25)	–	Osman et al., 2015
	Companion animals	Kennel dogs	Microscopy	126/305 (41)	–	Heilmann et al., 2018
Italy	Companion animals	Domestic and stray cats	IF, PCR	108 (6.1)	A, F	Paoletti et al., 2011
	Captive non-human primates	*Lemur catta*	Microscopy, PCR	8/17 (47,0)	–	Berrilli et al., 2011
	Pet and captive birds	Several species	Microscopy, PCR	4/146 (5.3)	A	Papini et al. 2012
	Pet rodent	*Chincilla lanigera*	IF, PCR	41/104 (39.4)	B, C	Veronesi et al., 2012
	Companion animals	Horses	PCR	38/431 (8.6)	B1, A1, E	Traversa et al., 2012
	Livestock	Cattle	ELISA, PCR	503 (32.2)	A, E	Geurden et al., 2012
	Companion animals	Owned dogs	ELISA + PCR	239 (3.8)	A, C	Riggio et al. 2013
	Companion animals	Owned cats	ELISA + PCR	81 (1.2)	A, C	
	Companion animals	Stray cats	ELISA	139 (2.9)	–	Spada et al., 2013
	Companion animals	Dogs	Microscopy + PCR	172/655 (26.3)	D, C, AII	Pipia et al., 2014
	Companion animals	Owned dogs	ELISA + PCR	253 (16.05–25.58)	C, D	Zanzani et al., 2014a
	Companion animals	Owned cats	ELISA, PCR	156 (22.47–36.84)	A, D	
	Companion animals	Dogs	Microscopy, ELISA	50/463 (11.6)	–	Zanzani et al., 2014b
	Companion animals	Owned cats	PCR	11/146 (7.5)	F, C	Mancianti et al., 2015
	Companion animals	Kenneled and owned dogs	Microscopy, PCR	41/502 (8.2)	C, D	Paoletti et al., 2015
	Companion animals	Kenneled dogs	Microscopy	48/218 (15.1)	–	Simonato et al., 2015
			PCR	165/285 (57.9)	C, D	
	Wild ruminants	Chamois	IF, PCR	7/157 (4,45)	AI, AIII, E	De Liberato et al., 2015
	Companion animals	Cats	IF	52/267 (19.5)	–	Veronesi et al., 2016
	Companion animals	Owned dogs	Microscopy	58/619 (9.4)	–	Tamponi et al., 2017
		Owned Cats	Microscopy	29/343 (8.5)	–	
	Companion animals	Dogs in public areas	Microscopy	8/705 (1.1)	–	Simonato et al., 2017
			PCR	204/705 (28.9)	C, D, B	
	Companion animals	Dogs (Animal Assisted Intervention)	Microscopy	18/74 (24.3)	–	Gerardi et al., 2018
	Companion animals	Kennel dogs	ICT, PCR	31/639 (4.8)	A	Sauda et al. 2018
	Companion animals	Household and Shelter dogs	Microscopy	159/2275 (7.0)	–	Scaramozzino et al., 2018
	Companion animals	Stray and shelter dogs	IF, PCR	56/262 (21.4)	C, D	De Liberato et al., 2018
	Wild animals	Wolves	PCR	1/20 (5.0)	–	Di Francesco et al., 2019
	Captive animals	Several species	Microscopy	1/30 (3.3)	–	Capasso et al., 2019
	Companion animals	Shelter cats	ICT	14/132 (10.6)	–	Sauda et al., 2019
	Companion animals	Dogs with lymphoma	ICT	6/30 (20.0)	–	Cervone at al, 2019
	Wild animals	Foxes	ICT	1/71 (1.4)	–	Papini and Verin, 2019
	Wild animals	Porcupine	Microscopy, PCR	25/52 (50.0)	B1, AII, BIV	Coppola et al., 2020
	Companion animals	Chronic enteropathy dogs	Microscopy, ICT, PCR	16/47 (34.0)	C, D	Perrucci et al., 2020
	Companion animals	Imported puppies	Microscopy, IFA	123/256 (48.5)	–	Cocchi et al., 2021
		Dog carcasses		6/62 (8.70)	–	
		Kittens (Cats)		0/62	–	
	Companion animals	Cats	IF, PCR	46/133 (35.3)	AI, AII, A3, B	Guadano Procesi et al., 2022
	Wild animals	Eurasian badger	ICT	21/43 (48.8)	AII, B	Maestrini et al., 2022
Slovenia	Livestock	Cattle	IF	104/391(26.60)	E	Van Lith et al., 2015
	Livestock	Sheep	IF	15/35 (42.86)	–	
	Livestock	Goats	IF	1/9 (11.11)	–	
Croatia	Wild mammals	Ruminants, Carnivores	IF, PCR	200/832 (24)	A, AI AIII, B, C, D	Beck et al., 2011a
	Captive animals	Several species	IF, PCR	38/131 (29)	A, B, C, D	Beck et al., 2011b
	Companion animals	Dogs	PCR	96	C, D	Beck et al., 2012
	Wild animals	Wolves	Microscopy, ELISA	8/400 (2.1)	–	Hermosilla et al., 2017
	Wild animals	Wildcats	Microscopy, IF	6/34 (17.6)	–	Martinković et al., 2017

(*continued on next page*)

Table 3 (*continued*)

Country	Animal group	Species	Method/s used	Positive/total (%)	Assemblage/sub-assemblage*	Refs.
	Companion animals	Dogs and cats	IF	1394/5387 (25.88)	–	Plutzer et al., 2018
Bosnia Herzegovina	Wild animals	Foxes	Microscopy, IF	4/123 (7.32)	–	Hodžić et al., 2015
	Companion animals	Dogs	Microscopy, IF	(6.60–100)	–	Plutzer et al., 2018
	Companion animals	Dogs	Microscopy, IF	212 (15.57)	–	Omeragić et al., 2021a
Albania	Companion animals	Shelter and household dogs	ELISA	214/602 (35.5)	C, D	Shukullari et al. 2013
	Companion animals	Cats	ELISA	17/58 (29.3)	–	Knaus et al., 2014
	Companion animals	Dogs	ELISA	159/602 (26.4)	–	Shukullari et al., 2015
Greece	Captive animal	Mara (*Dolichotis patagonum*)	Microscopy	1	–	Tahas and Diakou, 2013
	Livestock	Lambs	IF	160/429 (37.3)	A, E	Tzanidakis et al., 2014
	Livestock	Goat kids	IF	103/255 (40.4)	A, E	
	Companion animals	Foals	IF, PCR	22/190 (11.6)	A, B, E	Kostopoulou et al., 2015
	Companion animals	Dogs	IF, PCR	879 (25.2)	C, D, A	Kostopoulou et al., 2017
	Companion animals	Cats	IF, PCR	264 (20.5)	A, F	
	Companion animals	Cats	Microscopy	3/118 (2.5)	–	Giannelli et al., 2017
	Companion animals	Cats	Microscopy	26/1150 (2.3)	–	Symeonidou et al. 2018
	Livestock farms	Sheep farms	Microscopy	106/325 (32.6)		Lianou et al., 2022
		Goat farms		40/119 (33.6)		
Turkey	Companion animals	Dogs	Microscopy, PCR	473 (18.8)	AIII and BIV	Gultekin et al., 2017
	Companion animals	Horse	PCR	25/150 (16.6)	A	Demircan et al., 2019
	Livestock	Calves	Microscopy, PCR	27/198 (23.28)	A3	Kumar, 2020
Cyprus	Companion animals	Cats	Microscopy	12/185 (6.5)	–	Diakou et al., 2017
Israel	Companion animals	Dogs	PCR	74/302 (24.5)	C, D	Salant et al., 2020
	Companion animals	Dogs	ICT	19/163 (11.7)	–	Kuzi et al., 2020
Egypt	Livestock	Calves, dairy cattle	PCR	4/18 (22.2)	AI, E	Amer, 2013
	Livestock	Cattle	ICT, PCR	40/593 (6.7)	A, E	Helmy et al., 2014
		Buffaloes		10/211 (4.7)		
	Livestock	Dairy cows, calves	Microscopy, PCR	4/46 (8.7)	E	Abdel-Moein and Saeed, 2016
	Livestock	Calves	PCR	33/248 (13.3)	A, AII, E	Naguib et al., 2018a
	Livestock	Buffaloes, cows, sheep, goats	Microscopy	48/165 (29)	–	Shehab et al., 2021
	Captive animals	Several species	Microscopy, PCR	7/77 (9.09)	–	Kamel et al., 2021
Algeria	Livestock	Calves	PCR	28/102 (27.45)	A, AII, E	Baroudi et al., 2017
	Livestock	Lambs	IF, PCR	23/83 (28%)	A, D, E	Sahraoui et al., 2019
Morocco	Companion animals	Dogs	Microscopy	21/291 (7.2)	–	Idrissi et al., 2022

* Single or mixed genotypes; ** Fecal samples include Madrid zoo; "–" Not applicable, not investigated or not reported; °Pools; IFA: Immunofluorescence assay; ICT: Immunochromatographic test; ELISA: Enzyme linked immunosorbent assay; PCR: polymerase chain reaction.

Table 4
Giardia duodenalis cysts/DNA (Number of positive samples/number of examined samples (%)) in soil and fresh produce, in the Mediterranean drainage Basin. Results are presented in chronological order (2011–2022) according to the country.

Country	Source	Sample matrix	Method/s used	Positive/total (%)	Assemblage/sub-assemblage*	Refs.
Spain	Vegetables	Oak leaf lettuce, Romaine lettuce, Iceberg lettuce	PCR	30/129 (23.3)	–	Trelis et al., 2022
Italy	Vegetables	Ready to eat mixed salads	Microscopy, PCR	4/648 (0.6)	A	Caradonna et al., 2017
	Vegetables	Ready to eat mixed salads	Microscopy, PCR	25/72** (4.6)	AI B, E	Barlaam et al., 2022
	Berries	Blueberries, blackberries, raspberries				
Bosnia Herzegovina	Soil and vegetation	Soil and Vegetation	Microscopy, IFA	82/1,618 (5.06)	–	Omeragić et al., 2021
Syria	Vegetables	Radish, spearmint, lettuce, coriander, parsley	Microscopy	18/137 (13.13)	–	Alhabbal 2015
	Vegetables	Lettuce, parsley spearmint, radish, arugula watercress	Microscopy, PCR	17/128 (38.6)	–	Al Nahhas and Aboualchamat, 2020
Egypt	Soil	Soil	Microscopy	84/1070 (7.9)	–	el-Beshbishi et al., 2005
	Vegetables	Carrot, coriander, cucumber, pepper, parsley, radish, tomato	Microscopy	8/98 (8.2)	–	Hassan et al.,2012
	Vegetables	Rocket, lettuce, parsley, green onion, leek	Microscopy	19/300 (6.7)	–	El Said Said, 2012
	Vegetables	Lettuce	Microscopy	16/101 (15.8)	–	Eraky et al., 2014
		Watercress		13/116 (11.2)		
		Parsley		12/102 (11.8)		
		Greek onion		4/103 (3.9)		
		Leek		2/108 (1.9)		
Morocco	Vegetables	Carrot, coriander, lettuce, parsley, radish	PCR	6/132 (4.5)	–	Berrouch et al., 2020
	Soil	Wastewater-irrigated soil	Microscopy	(56–66.7)	–	Amahmid et al. 2022
	Vegetables	Coriander	PCR	3/51		Berrouch et al., 2022
		Persley		1/50		

*Single or mixed genotypes; "-" Not applicable, not investigated or not reported; **Pools of 324 packages each; IFA: Immunofluorescence assay; PCR: polymerase chain reaction.

water supplies, also for the often illegal use of their manure for fertilisation. Although ungulates-related assemblage E has been detected in livestock, they also shed zoonotic assemblages thus representing a reservoir of *G. duodenalis,* with the potential to cause disease in humans. It is noteworthy the detection of dog-specific assemblage (D) found in lambs in Algeria (Sahraoui et al., 2019); large-scale studies are needed to better understand *Giardia* circulation assemblages not adapted to ruminants.

4.3. Soil and fresh produce contamination

As shown in Table 4, data on contamination by *G. duodenalis* in soil and/or vegetables are available from five Mediterranean countries, i.e., Spain, Italy, Bosnia Herzegovina, Greece, Syria, Egypt, Morocco. Overall, over 4600 samples were examined, by different techniques.

The most investigated matrices were vegetables with prevalence of contamination up to 38.6% (Al Nahhas and Aboualchamat, 2020). Among fresh produce, worthy of note is the prevalence of up to 4.6% in ready-to-eat salads and berries in Italy (Caradonna et al., 2017; Barlaam et al., 2022). As to soil, this matrix was studied in three countries (Bosnia Herzegovina, Egypt and Morocco) with prevalence up to 66.7% in soil irrigated by wastewater (Amahmid et al., 2022).

Genotyping data are available only in Italy where several *Giardia* assemblages (A, B and E) were detected in fresh produce (Caradonna et al., 2017; Barlaam et al., 2022).

The recurrent shedding of *Giardia* by infected hosts justifies the water and consequently the soil and produce contamination. *Giardia* cysts can persist for months in soil under suitable moisture and temperature conditions while retaining their infectivity (Resi et al., 2021; Conners et al., 2021; Krumrie et al., 2022). It is impressive the spread of contaminated marketed vegetables/fruits, which are commonly eaten raw. The presence of *Giardia* in ready-to-eat salads and berries is of concern, especially if we consider that they have been shown to contain the zoonotic assemblage A (Caradonna et al., 2017; Barlaam et al., 2022). The detection of Assemblage E which is considered livestock-specific may be related to the use of manure as fertilizers or contaminated water. Its detection in humans arises the still open question on its zoonotic potential.

4.4. Water contamination

As shown in Table 5, data on contamination by *Giardia* in waters of different origins are available from ten out of 22 countries bordering the Mediterranean Basin: Spain, France, Italy, Greece, Turkey, Lebanon, Israel, Egypt, Tunisia, Algeria. Overall, over 2480 water samples were examined (most of them from Egypt and Spain) by different techniques. *G. duodenalis* have been reported in several kinds of waters (e.g. wastewater, surface, drinking, recreational). *Giardia* cyst positivity indicates that from 5.2% to 100% of waters are contaminated. The most investigated water sources were from wastewater treatment plants in particular in raw water/sludge with prevalence ranging from 12.3% in Greece (Spanakos et al., 2015) to 100% in Spain, Israel and Tunisia (Alonso et al., 2011; Ramo et al., 2017b; Nasser et al., 2020; Taran-Benshoshan et al., 2015; Sabbahi et al., 2018).

Drinking water was investigated in Spain, Greece, Lebanon and, in particular, in Egypt (Dyab et al., 2015; Sakran et al., 2017; Hamdy et al., 2019; Abd El-Latif et al., 2020; Elmehy et al., 2021) and Tunisia (Ayed et al., 2018) where higher prevalence up to 92% were recorded in drinking water stored in private cisterns in rural areas.

Data on surface water are available for irrigation channels and ponds from Spain, Greece, Turkey and Egypt, with prevalence values ranging from 15.6% (Gracenea et al., 2011) to 100% (Moreno et al., 2018). As for recreational waters, only one study on *Giardia* cyst contamination in swimming pools from Egypt was retrieved (Abd El-Salam et al., 2012), showing a prevalence of 6.6%.

Typing data were from six countries: Spain, Italy, Greece, Israel, Egypt, and Tunisia. Different assemblages/sub-assemblages, mostly related to the two potentially zoonotic groups A (AI, AII) and B (BIII, BIV) and several mixed infections were detected in wastewater (Spain, Italy, Greece, Israel, and Tunisia) (Ramo et al., 2017b; Benito et al., 2020; Moreno-Mesonero et al., 2022; Marangi et al., 2015; Ligda et al., 2020a; Nasser et al., 2020; Taran-Benshoshan et al., 2015; Ben Ayed et al., 2012), but also in tap water/drinking water (Greece and Egypt) (Ligda et al., 2020b; Hamdy et al., 2019; Abd El-Latif et al., 2020) and surface waters (Spain, Greece) (Moreno et al., 2018; Ligda et al., 2020b). Livestock-related assemblage E was also identified in wastewaters and surface waters in Spain, Greece and Tunisia (Ramo et al., 2017b; Ligda et al., 2020b; Ben Ayed et al., 2012).

The high levels of water contamination by *Giardia* cysts reflects the discharge of untreated sewage and the urban and/or rural land drainage polluted by human and/or animal faeces. However, the burden of these sources differs among the diverse watersheds and it is strongly dependent on the ecological, climatic and social factors characterising the area. Overall, data on water contamination in the Mediterranean Sea draining areas are much more available for the northern Mediterranean countries (Region I) than for the other Mediterranean regions, in particular, the Region III (see Fig. 1). However, watersheds in these areas, particularly in southern ones, have generally a lower streamflow input into the Sea, due to lower precipitation but also to the absence of large rivers, with the exception of the Nile. Surprisingly, within the period analyzed, only one survey on the presence of *Giardia* in waters from the French Mediterranean region is available, although the Rhone river system is one of the major drainage basins of the Mediterranean Sea.

4.5. What salient issues?

This review highlights that a high level of *Giardia* cyst contamination is recorded in the terrestrial environment. Data analysis obtained from the 25 countries - which can contribute in different ways to the contamination of the Mediterranean Sea - showed that *G. duodenalis* is very widespread among several animal species and humans. Zoonotic or species-specific assemblages circulate on land and are discharged into the sea water thus contaminating the marine inhabitants. However, despite the data provided according to the literature, some issues should be highlighted: *i)* it was sometimes difficult to correctly and exactly identify/delimit the areas strictly included in the drainage basin of the Mediterranean Sea (as shown in Fig. 1); *ii)* the multiple methods used for the detection of *Giardia* cysts in the investigated samples (e.g. light microscopy techniques, direct immunofluorescence, immunochromatographic tests, enzyme immunoassay, enzyme-linked immunosorbent assay, PCR-based molecular techniques, and others) make it difficult to compare the results, and to deduce a realistic picture of the contamination levels between and within countries and thus the real impact of such contamination on the Mediterranean Sea; *iii)* the role of other countries where few or no studies are available, such as Gibraltar, Montenegro, North Macedonia, Cyprus, Lebanon, Palestine (Gaza Strip), Libya, remains undefined.

5. *Giardia* survival in waters

The importance of *Giardia* dissemination from land to sea is highly related to cyst survival in waters. Indeed, in aquatic environments the survival seems to be strictly related to water temperature rather than to other parameters such as oxygenation, pH, or turbidity (Bingham et al., 1979; Faubert et al., 1986; de Regnier et al., 1989; Olson et al., 1999). Using excystation as criterion for viability, *Giardia* cysts kept at −13 °C showed almost complete loss of viability after freezing and thawing (though a level of viability <1% persisted for at least 14 days) while they were able to retain their viability for 77 days, 5–24 days, and 4 days when stored at 8 °C, 21 °C, and 37 °C, respectively (Bingham et al., 1979). In a study where *Giardia muris* was used as a model, cysts lost

Table 5
Giardia duodenalis cysts/DNA (Number of positive samples/number of examined samples (%)) in rivers, lakes, ground water, drinking water, wastewater in the Mediterranean drainage Basin. Results are presented in chronological order (2011–2021) according to the country.

Country	Source	Matrix sample	Method/s used	Positive/ total (%)	Cyst quantification	Assemblages/sub-assemblages*	Refs.
Spain	Wastewater treatment plants	Raw sewage samples	IFA, PCR	14/14 (100)	38–145 cysts/100 ml^{-1}	–	Alonso et al., 2011
	Irrigation channels	–	Microscopy	5/32 (15.6)	2–5 cysts/l	–	Gracenea et al., 2011
	Waste stabilisation pond system	Raw wastewater	IFA	26/26 (100)	67.1 cysts/l	–	Reinoso et al., 2011
		Final effluent		(73)	<1 cyst/l		
	Wastewater treatment plants	Raw sludge	IFA	26/30 (86.6)	1–248 cysts/g	–	Amorós et al., 2016
		Treated sludge		25/30 (83.3)			
	Drinking water treatment plants	Influent raw water	IFA	12/20	125 ± 241 cysts/ 100 l	–	Ramo et al., 2017a
		Effluent finished water		9/20	37 ± 41 cysts/100 l		
	Urban wastewater treatment plants	Influent wastewater	IFA	92/92 (100)	3247 ± 2039 cysts/l	AII, B, E	Ramo et al., 2017b
		Effluent wastewater		92/92 (100)	50 ± 28 cysts/l		
		Dewatered sewage sludge		92/92 (100)	20–593 cysts/g		
	Surface irrigation water samples	–	IFA, NGS	3/3 (100)	180–768 cysts/l	A, B	Moreno et al., 2018
	Drinking water treatment plants	Raw surface water from river	Microscopy	11/12 (91)	4.6 cysts/l	–	Pascual-Benito et al., 2020
		Raw water from river after reservoir system		(62.5)	0.78 cysts/l		
	Wastewater treatment plants	Influents	Microscopy, PCR	3/5	–	B	Benito et al., 2020
		Effluents		1/5			
	Urban wastewater treatment plants	Secondary treatment	PCR, NGS	3/4	–	A	Moreno-Mesonero et al., 2022
		UV tertiary treatment		3/4			
France	Roof Runoff Water	Tank		1/14	0.0050/100 mL	–	Vialle et al., 2012
Italy	Zootechnical wastewater	Primary effluents	IFA, microscopy	–	$2.4 \times 10^3 \pm 1.2 \times 10^3$ cysts/l	–	Bonadonna and Briancesco, 2011
		Phytodepurated effluents		–	$3.0 \times 10^1 \pm 1.6 \times 10^1$ cysts/l		
	Water treatment plants	–	PCR	16/119 (13.4)	–	A	Marangi et al., 2015
	Wastewater treatment plants	Influent	IFA	–	$4.1 \pm 6.2 \times 10^4$ cysts/l	–	De Sanctis et al., 2016
		Effluent			5.2 ± 6.2 cysts/l		
	Wastewater treatment plants	Wastewater	IFA	–	$1.3 \pm 1.6 \times 10^3$ cysts/l	–	De Sanctis et al., 2017
		SBBGR effluent			$2.9 \pm 3.6 \times 10$ cysts/l		
		SF effluent			0.7 ± 0.7 cysts/l		
Greece	Wastewater treatment plants	–	IFA	9/73 (12.3)	–	–	Spanakos et al., 2015
	Wastewater treatment plants	–	IFA, PCR	13/18 (72.0)	3.3 ± 3.4 cysts/l	AII	Ligda et al., 2020a
	Surface/drinking water	Raw surface water from rivers	IFA, PCR	90/136 (66.2)	4.7 ± 8.4 cysts/l	A, AI, AII, A4, E	Ligda et al., 2020b
		Irrigation canals		(50)	0.5 ± 0.5 cysts/l		
		Water production company		(167)	0.1–5.3 cysts/l		
Turkey	Waters	Irrigation waters	Microscopy	12/36 (33.33)	–	–	Sağlam et al., 2022
Lebanon	Drinking water	Vendor	IFA	16/32 (50)	–	–	Khoury et al., 2016
		School		2/5 (40)			
		Well		6/11 (54)			
		Shop		2/4 (50)			
		Municipality		2/3 (67)			
Israel	Wastewater treatment plants	Raw wastewater	IFA, PCR	42/42 (100)	3685 cysts/l	A, B	Nasser et al., 2020
	Wastewater treatment plants	Raw wastewater	IFA	43/43 (100)	3689.71 cysts/l	–	Taran-Benshoshan et al., 2015
		Secondary. effluents		61.9%	5.18 cysts/l		
		Tertiary effluents		76%	0.93 cysts/l		
Egypt	Swimming pools	–	Microscopy	2/30 (6.6)	–	–	Abd El-Salam et al., 2012
	Waters	River Nile	Microscopy	3/8 (37.5)	–	–	Khalifa et al., 2014
		Ponds		2/8 (25.0)			
		Canal		3/8 (37.5)			

(*continued on next page*)

Table 5 (*continued*)

Country	Source	Matrix sample	Method/s used	Positive/ total (%)	Cyst quantification	Assemblages/sub-assemblages*	Refs.
	Drinking water supplies	–	Flow cytometry	14/48 (29.2)	837.1 cysts/l (winter) 1,066.3 cysts/l (summer)	–	Dyab et al., 2015
	Domestic wastewater	–	Microscopy	8/11 (72.7)	–	–	Tawfik et al., 2015
	Waters	Source	Microscopy, IFA, Flow cytometry	30/108 (27.8)	–	–.	El-Kowranyet al., 2016
		Plant		5/108 (4.6)			
		Tap water		8/108 (7.4)			
	Waters	Tap water	Microscopy	8/65 (12.3)	–	–	Sakran et al., 2017
		Tanks		5/30 (16.7)			
		Ground water	Microscopy	22/245 (9)	–	–	Elfadaly et al., 2018
	Drinking water	Tap water	Microscopy, PCR	20/80 (25)	–	AII, BIII, BIV	Hamdy et al., 2019
	Waters	Raw water	Microscopy, PCR	10/10 (100)	–	AII	Abd El-Latif et al., 2020
	Drinking water	Water tanks	Microscopy, IFA,	(73.43)	–	–	Elmehy et al., 2021
		Tap water	Flow cytometry, PCR				
Tunisia	Wastewater treatment plants	Raw water	PCR	47/110 (42.7)	–	A, B, E	Ben Ayed et al., 2012
		Treated water		15/110 (13.6)			
		Sludge samples		3/12 (25)			
	Drinking water	Private cisterns	Microscopy	36/39 (92)	13–393 cysts/l	–	Ayed et al., 2018
	Wastewater treatment plants	Raw water	Microscopy	20/20 (100)	–	–	Sabbahi et al., 2018
		Treated water		16/20 (80)			
	Wastewater treatment plants	dried sewage sludge	Microscopy	116/116 (100)	2.25 cysts/100 g	–	Sabbahi et al., 2022
Algeria	Wastewater treatment plant	Raw water	Microscopy	–	26 ± 22 cysts/l^{-1}	–	Hamaidi-Chergui et al., 2019

*Single or mixed genotypes; "–" Not applicable, not investigated or not reported; IFA: Immunofluorescence assay; PCR: polymerase chain reaction; NGS: next generation sequencing.

their infectivity for mice after 30 days at 4 °C either in tap water or in well water (Faubert et al., 1986). In autumn, *G. muris* cysts in lake water remained viable for up to 28 days when suspended at about 4.5 m deep, whereas they remained viable for up to 56 days at a depth of about 9 m. Cysts exposed to river water remained viable for up to 28 days whereas they were non-viable after exposure to tap water for 14 days. In winter, cysts showed no signs of viability after 84 days both in lake water and in river water as well as after 14 days in tap water (De Regnier et al., 1989). In winter (temperature range = 1 to 7 °C) in an aquatic environment in Norway, after one month, no cysts with apparently viable morphology were detected, suggesting that *Giardia* cysts cannot overwinter in Norwegian environmental conditions (Robertson and Gjerde, 2006). Conversely, lower river water temperatures (1.1 to 6.7 °C) and lower air temperatures (–0.1 to 4.5 °C) significantly increased the odds of *Giardia* presence by four times compared to higher water and air temperatures in Canada (Masina et al., 2019).

In combination with temperature, exposure to natural sunlight UV irradiation can also play a role in the survival of *Giardia* cysts in water. In experimental conditions, *G. muris* cysts lost their infectivity to mice after exposure to simulated global solar irradiation for 4 h at a constant temperature of 40 °C (McGuigan et al., 2006). The synergistic effect of UV radiation and the heat produced by the sun inactivated 99.9% of *G. duodenalis* cysts from human stool samples, suspended in tap water, when the water temperature reached 56 °C after exposure to direct sunlight for 7 h (Mtapuri-Zinyowera et al., 2009).

Almost nothing is known about the persistence of *Giardia* cysts in marine environments. The survival of *G. muris* cysts in marine water varied according to sunlight, salinity, and water quality. In particular, cysts held in the dark survived much longer (77 h) than those held in the direct sunlight (3 h). In addition, salinity played a significant role in inactivation of *G. muris* as cysts held at 35 ppt (parts per thousands) salinity were inactivated more rapidly than those held in canal water (28 ppt). Together, both salinity and light inactivated the cysts within 3–6 h. Interestingly, cysts survived longer in polluted canal water (121.3 h) than in marine water in the dark (97.1 h), suggesting that salinity was more responsible than water quality for their inactivation (Johnson et al., 1997).

6. Effects of climate change

Climate change consists of two main components that can vary at different times and places: the global warming due to the increasing concentration of greenhouse gases such as carbon dioxide, methane, nitrous oxide, ozone and fluorinated gases and the consequent change of the hydrological cycle with increasing evaporation and intensification of the Earth's water cycle (Ali et al., 2022). Both factors will cause some land areas to dry out, but will also contribute to more frequent and intense storms in other areas. Indeed, climate change is generally expected to worsen the frequency, intensity and impacts of extreme weather events (increased risk of floods, droughts, and heat waves).

Given its status as a climate change hotspot, the Mediterranean is a region that needs special attention. The Intergovernmental Panel on Climate Change identified the Mediterranean ecosystems as being among those most affected by the effects of the atmosphere's rising concentration of greenhouse gases in its Fifth Assessment Report (https://eo4society.esa.int/regional-initiatives/mediterranean-regional-initiative/mediterranean-regional-initiative-overview/). Recent satellite observations and in-situ oceanographic and meteorological records indicate that the Mediterranean Sea has warmed by an estimated 0.6 °C to 1 °C over the past three decades (Lopez-Garcia, 2021). It is known that 2 °C global warming would reduce rainfall by around 10–15% and an increase between 2 °C and 4 °C would reduce rainfall by up to 30% in Southern Europe.

By 2100, it is anticipated that water temperatures will increase by 1.8 °C to 3.5 °C, with hotspots in Spain and the Eastern Mediterranean (https://www.unep.org/unepmap/resources/factsheets/climate-cha

nge).

Climate change has a direct impact on parasite life cycles, increasing or decreasing development and survival of parasite stages in the environment and influencing the biology of their hosts (Mignatti et al., 2016; Knapp-Lawitzke et al., 2016). The intensity of rainfall favours the spread of resistant forms through contaminated waters (Jiménez et al., 2010). Drought, on the other hand, reduces the survival of parasitic forms but the consequent evaporation increases their concentration in water. The growing circulation of low quality waters raises the risk of outbreaks.

The protozoan pathogen *Giardia* (like other intestinal protist parasites, i.e., *Cryptosporidium*) is particularly affected by these changes (Lal et al. 2013). Climate change may affect the occurrence, distribution and transmission of *G. duodenalis* in the Mediterranean countries in different ways (Ahmed et al., 2018). The spread of cysts in the environment is influenced by seasonal rainfall, which carries the cysts from land to water, including drinking water, with risk of waterborne giardiasis, and affects the flow of rivers leading to the sea contamination with this flagellated protozoan. Climatic conditions can also affect the geographic distribution and population density of humans and animal species that serve as suitable hosts and play an important role in the occurrence, distribution and transmission of *Giardia*. Both outbreaks and sporadic cases of giardiasis have been linked to a variety of weather elements that may be affected by climate change (Young et al., 2015). A large seasonal increase in human giardiasis has been reported during the summer months in the developed countries (Brunn et al., 2019). A positive association of human giardiasis cases with water levels and flow rates has been demonstrated in relation to the increased cyst concentration in rivers (Brunn et al., 2019); resuspension of enteric protozoa from river sediments can be assumed during periods of high water flow, such as during storms, as shown for other faecal microorganisms, e.g. *E. coli* (Dorner et al. 2006).

The contamination and the risk of transmission for this and other pathogens is worsened by various socio-anthropological aspects - linked to climatic changes -, such as *i)* the greater number of people living in urban areas, particularly in the Mediterranean countries; *ii)* new food habits, i.e. higher consumption of fresh produce - locally produced or imported; *iii)* increased colonization by wild animals of the urban and periurban environment (Pozio, 2020).

7. Present and future scenario, challenge and perspectives

Countries bordering the Mediterranean Sea share common identities but also display an extreme disparity in wealth, resources and health. In recent decades, the Mediterranean region has undergone an extensive and profound urban transformation linked to population growth, increasing rate of urbanization, agriculture, tourism, and profound social and economic changes. Of the total 46,000 km of coastline, 25,000 km are urbanized and 250 million people (55% of the total population) reside in coastal areas, of which 56% (120 million) in the southern regions of the Mediterranean. The highest concentration of population in coastal areas is observed in the eastern Aegean Levantine region, in the western Adriatic Sea, and in the Nile Delta (European Environment Agency, 2020).

As a result, a huge amount of natural wetland habitats has been lost (Fluet-Chouinard et al., 2023). (https://www.unep.org/unepmap/news/news/working-healthy-coastal-wetlands-mediterranean).

As a further consequence of this high degree of anthropization, the Mediterranean Sea receives over 10 billion tonnes of industrial, animal and urban waste per year with little or no purification (De Stefano, 2004; WTO, 2020).

An intriguing and unexplored aspect concerns the association of terrestrially derived zoonotic protozoan parasites with microplastics in seawater. Plastics, especially microplastic, are widely recognized as a pervasive marine anthropogenic pollutant. They can be ingested by fish or filtered by marine invertebrates such as shellfish, including those habitually used for human consumption. As demonstrated by Zhang et al. (2022), parasites, including *Giardia*, are likely associated with microplastics in contaminated seawater; as a consequence, plastic particles may result in a concentration and/or dispersal of pathogens to locations distant from their original terrestrial source, thus represent a novel pathway of the transmission of pathogens in the marine environment, with important implications for wildlife and human health.

The Mediterranean population is predicted to increase to 572 million by 2030 and that of the coastal regions is expected to increase by over 180% by 2100 (Reimann et al., 2018). This entails an ongoing intensification of agricultural practices (crops and livestock), which will, on the one hand, result in greater use of water for irrigation and, on the other hand, have negative effects on water resources, biodiversity, and the health of the landscape.

The reduction and loss of wetlands will further exacerbate the impact caused by the coastal development of human activities, because coastal ecosystems can remove pathogens from contaminated water runoff and mitigate their effects before discharge to the sea (Shapiro et al., 2010; Klohmann and Padilla-Gamiño, 2022).

At the same time, the Mediterranean Sea traffic - the busiest shipping lanes in the world - is forecasted to grow by 4% annually until 2025 (https://eo4society.esa.int/regional-initiatives/mediterranean-regional-initiative/mediterranean-regional-initiative-overview/).

All these factors, including the impacts of climate change, have the potential, individually and in synergy, to increase land-sea water flow and thereby increase the transport of *Giardia* cysts and other land-borne pathogens and pollutants into the Mediterranean Sea.

It should be taken into account that Spain, Turkey, Italy, France, and Egypt are the countries with the highest anthropogenic impact (Sharma et al., 2021; Cantasano 2022). Moreover, it has been shown that 12 ports of the Adriatic Sea are responsible for the highest faecal contamination (Luna et al. 2019).

To reduce sea feacal contamination it is crucial to monitor hosts and water pollution in order to understand the pressure of parasite contamination of the Mediterranean Basin. Therefore, water monitoring systems, improving hygiene practices, and the use of properly purified water are essential (Omarova et al., 2018). In this perspective, it becomes imperative *i*) to apply, implement and standardize the legislative measures for microbiological detection in water and wastewater, adding *Giardia* - as well as other pathogenic protists - to the list of contaminants; ii) to check the hygienic-sanitary quality of shellfish, whose role as bio-indicators of water contamination is accepted and confirmed worldwide (Giangaspero et al., 2019; Bigot-Clivot, 2022); *iii*) to monitor the degree of environmental pollution of the waters discharged into the sea (faecal contamination from urban and/or livestock waste); *iv*) to investigate the origin of protozoan isolates (animal and/or human); *v*) to monitor the possible presence of illegal urban and/or livestock discharges that flow into the main watercourses directed towards the sea; *vi*) to evaluate the removal efficiency of highly resistant protozoans from wastewater treatment plants. It is known that treatment plants are sometimes even inactive due to operating costs, as has been seen, particularly, in some areas of the Mediterranean Basin (Giangaspero, pers. com.). The implementation of sewage treatment plants must take into account technological advancement, and only the application of multi-barrier systems can ensure a more effective water purification.

Moreover, actions aimed to reduce impermeable land surfaces and maintain or restore coastal wetlands could mitigate the impact of climate variability, human and animal population growth, and land-use changes on the flow of *Giardia* from land to sea. Although many countries have worked over the past 40 years to put in place cooperation mechanisms finalized to manage and protect the Mediterranean, ever more intensive efforts need to be done in the future, even with regulations shared between all the countries bordering the Mediterranean Sea, to safeguard this special and at the same time extremely delicate coastal and marine environment. This review calls for more integrated marine environmental strategies to carry out regular protection programs supported by national and international authorities responsible for the

conservation of this threatened and vulnerable sea.

Safeguarding the Mediterranean can only be achieved with a "One Health" approach in which ecologists, biologists, epidemiologists, physicians, veterinarians and professionals together with politicians and society as a whole, must collaborate and face this challenge.

CRediT authorship contribution statement

FB, RAP, AG: Conceptualization, Data curation, Investigation, Methodology, Writing - original draft, Writing review and editing, Funding acquisition. **GN, AB, AP:** Investigation, Data curation, Writing original draft. **IGP:** Data curation, Writing review and English and Reference supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data used are available in the manuscript and in the additional files.

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