Toxoplasma gondii in Marine Life of Italian Coasts

Subjects: Parasitology
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Coastal areas of Italy experience high anthropogenic pressure, with a population density estimated to be 360 people per km^2. This is correlated with the production of sewage or surface runoff of water contaminated with *Toxoplasma gondii* oocysts and other pathogens that can in turn enter the food chain and become a public health concern.

Toxoplasma gondii detection environment Italian coast

1. Distribution of *T. gondii* in Italy

In Europe, animal husbandry biosecurity measures and good manufacturing practices in the meat industry have been considerably improved, shifting attention to the role of oocysts in the epidemiology of this parasite. In recent years, long-term observations of pregnant women showed that the infection cannot always be linked to known risk factors such as the consumption of cyst-contaminated meat. Environmental exposure to *T. gondii* oocysts can indeed be an important route for human infection [1]. More sensitive analytical methods have been developed for the detection of oocysts in soil and water samples, adding evidence on the spread of oocysts via shedding in the environment. The role of oocysts is no longer seen as secondary to tissue-cyst-based transmission.

Half of the game produced in Europe is estimated to be seropositive for *T. gondii*, and meat from game is identified as an important source of *T. gondii* infection for consumers, including venison and wild boar meat [2][3]. Moreover, atypical and recombinant genotypes have been detected in wild animals in northern and central Italy [4][5].

Different studies report the seroprevalence of *T. gondii* in livestock in different Italian regions. A recent study conducted in the north of Italy reports seropositivity in different animals: 42.9% in cats, 29.9% in sheep, 25% in roe deer, 21.8% in dogs, 18.7% in goats, 15.5% in wild boar, and 9.7% in pigs. The same study reports a seroprevalence of 20.4% on a screened population of 36,814 individuals, while, among pregnant women, toxoplasmosis was active in 0.39% of cases [6]. Overall, the seroprevalence among small ruminants in farms of central and northern Italy is 90.7% [7] and 68.4% in cattle farms [8]. In southern and insular regions of Italy, there is a similar epidemiological scenario, with a prevalence of 87% among small ruminants farmed in Sicily [9]. In Campania, a southern region, the prevalence among livestock is reported at 77.8% in sheep [10] and 13.7% in water buffalo [11]. A seroprevalence of 39.6% was found among wild boar in the south [12].

Most cats are infected by ingesting infected prey [13]. Up to one billion oocysts can be shed by a single cat over a 1–2-week period, and they can repeatedly shed oocysts during their lifetime [14]. According to a survey from FEDIAF [15], between 2020 and 2021, the total number of cats owned by Italian households increased by almost 27%, reaching a population of 10.2 billion. Official feline population data and cat censuses are difficult to gather since it is not always recognized as a public health concern. In Italy, cat registration and identification are carried out on a voluntary basis. A multi-center study carried out in 2021 including 987 cats distributed across the country estimated that 27.9% of cats were living in colonies, 69.2% had a private owner, and 2.9% were living in shelters [16]. Among owned cats, it has been shown that the majority are living in rural areas (67.8%), being kept outdoors and often in households with other pets [17]. The same survey estimated that 32% of cats in Italy do not attend a veterinary clinic [17]. However, few studies have been conducted at a national level to determine the *T. gondii* seroprevalence among domestic cats. In Europe, the prevalence among feral and stray cats ranges between 18.27% and 40.7%, varying with the age and lifestyle of the cat [18]. In Italy, a seroprevalence of 40.7% was estimated among stray cats, being higher in urban (45%) than suburban areas (35.7%) [19].

ISTAT reported a yearly average temperature of 15.6 °C for 2021, with an increase of +0.6 °C compared to the period 1981–2010 and +1 °C compared to the period 1971–2000. In total, the yearly precipitation accounted for 746 mm in 2021, but 2022 presented a very dry year, with 455 mm [20]. In the current year, precipitation has already reached 500 mm, and it is projected to be a rainy fall and winter. Climatic changes with periods of drought followed by intense periods of rain could facilitate the dispersion of oocysts, as previously stated.

2. *Toxoplasma gondii*: From Land to Sea

Anthropogenic pressure on the coastline increases pollution with sewage or surface runoff of water possibly contaminated with *T. gondii* oocysts. It has been demonstrated that the distribution of pet cats and feral cat colonies is linked to human settlements that can provide food and shelter, even when feral cats are not directly fed or taken care of [21]. This is probably the key factor in *T. gondii* epidemiology in marine environments, with feral and domestic cats as the sole source of oocysts [22]. After infection, a single cat can shed millions of oocysts within 1 week, and, as previously reported, they can remain viable in soil for up to one year [23].

Reports of infection among marine mammals are becoming more frequent worldwide [24][25][26][27][28]. Clinical symptoms and seroprevalence have been described in sea otters, seals, sea lions, manatees, walruses, and dolphins [24][25][26][27][28]. It was only in the early 2000s that it was possible to isolate *T. gondii* from brain and heart tissues of sea otters (*Enhydra lutris*) in the National Wildlife Health Center (NWHC) of Madison, Wisconsin (USA) [29]. In the waters of the European continent, *T. gondii* has been found in the common dolphin (*Delphinus delphis*), striped dolphin (*Stenella coeruleoalba*), common bottlenose dolphin (*Tursiops truncatus*), long-finned pilot whale (*Globicephala melas*), Risso's dolphin (*Grampus griseus*), harbor porpoise (*Phocoena phocoena*), humpback whale (*Megaloptera novaengliae*), bearded seal (*Erignathus barbatus*), harbor seal (*Phocavitulina*), ringed seal (*Pusa hispida*) and grey seal (*Halichoerus grypus*) [29].

Novel routes of oocyst transmission involving suspended microplastics have been found [30]. Parasites can associate with microplastics in contaminated seawater, suggesting that they may facilitate pathogen entry into marine food webs. Parasites could therefore be incorporated into aggregates of nanoparticles and ingested by filter-feeding marine invertebrates that may not be able to capture freely moving parasites [30]. Moreover, microplastics have the capacity to float or sink. In the first case, by floating on the sea surface, they can travel large distances, which may facilitate the dispersion of pathogens far from the areas from where they came. In the second case, sinking particles will accumulate in the benthos, where pathogens will concentrate and filter-feeding invertebrates are more likely to ingest them [31].

3. *Toxoplasma gondii* in the Italian Sea Environment

3.1. Marine Mammals

Toxoplasma gondii constitutes a major zoonotic agent and a significant cause of clinical disease in wildlife such as abortion, pneumonia, or encephalitis. Specifically, this parasite has been associated with neurological disease and encephalitis in cetaceans, becoming a primary neurotropic pathogen for striped dolphins [32][33][34][35]. A recent meta-analysis on marine species affected with *T. gondii* in Europe shows a total prevalence of 13%. The most affected are marine fissipeds (53.1%), followed by mollusks (26.4%), fishes (21.8%), cetaceans (14.8%), and pinnipeds (2.8%) [36].

The majority of data on the presence of *T. gondii* in Italian waters is related to stranded marine mammals. The International Pelagos Sanctuary, which occupies circa 90,000 km^2 of international waters between France, Italy, and the Principality of Monaco, is a valuable source of information on marine mammals. It was established in 1999 to protect cetaceans from the combined pressures of natural environmental fluctuations and human impacts. It constitutes the first transboundary marine protected area, being a region with a high biomass of diversified plankton that attracts all eight cetaceans present in the Mediterranean Sea. Stranded cetaceans have been registered and analyzed in recent decades in this area, providing information on the presence of *T. gondii* and other parasites affecting the health of marine mammals.

3.2. Fish

In Italy, some fish species, such as anchovies (*E. encrasicolus*), are consumed raw or following marination in lemon juice for a few hours, which is not enough to inactivate oocysts [37]. Moreover, most of the species consumed in Italy are demersal or benthopelagic, living on different types of seabeds (sand, mud, rocks, or seagrass beds). In this environment, *T. gondii* oocysts are more likely to settle, sometimes aided by aquatic invertebrates that facilitate settling and help benthos concentration.

Only one study has investigated the presence of *T. gondii* in fish from local markets, analyzing a total of 1293 individuals from 17 different species, pooled into 147 groups [22]. Samples were obtained by pooling intestines, gills, and skin/muscles. *T. gondii* DNA was found in 12 of 17 fish species tested with 32 positive samples out of 147 overall [22]. Of these samples, 16 were of the skin/muscle and 11 of intestines and gills [22]. Fish was purchased at MAAS (Sicily Agro-Food Markets), which is the biggest market in Sicily, and in other small-size fisheries that sell fish from the area FAO 37.2.2.

3.3. Shellfish

There have been four studies analyzing shellfish in Italy in the last ten years, one regarding crustaceans and three regarding bivalves. The study on crustaceans collected Atlantic blue crab (*Callinectes sapidus*) from Lesina Lagoon and analyzed hemolymph, gills, stomach, hepatopancreas, and gonads using PCR. *T. gondii* was mostly found in the gills (n = 4), hemolymph (n = 2), and stomach (n = 1) [38].

Of the three studies analyzing bivalves in Italy, two focused on Mediterranean mussels (*Mytilus galloprovincialis*). In the study by Marangi et al., 53 samples from Turkey and 60 from Foggia food markets (Italy) were analyzed using qPCR targeting the B1 gene [39]. While all the samples from Italy were negative, 7 out of 53 (13.2%) mussel DNA samples from Turkey tested positive for *T. gondii*, and the type I genotype was confirmed [39]. In the study by Santoro et al., they analyzed 382 samples of *M. galloprovincialis* sampled in seven production sites in the Gulf of Naples and 27 farmed Mediterranean mussels obtained from a mollusk depuration plant in Corigliano Calabro (Calabria region) [40]. Digestive glands were used for the detection of the *T. gondii* B1 gene using qPCR. *T. gondii* DNA was detected in 39 out of 382 (10.2%) Mediterranean mussels from 6 out of 7 sampling sites in the Gulf of Naples and 4 out of 27 individuals from the mollusk depuration plant in Corigliano Calabro [40].

Only one study analyzed edible farmed shellfish. The study collected a total of 1734 individuals divided into 62 pooled samples: 109 *Crassostrea gigas* (6 pools of gills), 660 *Mytilus galloprovincialis* (22 pools), 804 *Tapes decussatus* (28 pools), and 161 *Tapes philippinarum* (6 pools) [41]. *T. gondii* DNA was detected by both nested PCR and real-time FLAG assay in 2 pooled samples out of 62 (3.2%) [41].

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