Toxoplasma gondii in Foods

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Contributor: Pablo-Jesús Marín-García , Nuria Planas , Lola Llobat

Toxoplasma gondii is an obligate intracellular parasite that causes toxoplasmosis, with approximately one third of the population around the world seropositive. The consumption of contaminated food is the main source of infection. These include meat products with *T. gondii* tissue cysts, and dairy products with tachyzoites.

toxoplasmosis Toxoplasma gondii control food detection

1. Introduction

Toxoplasmosis is a zoonotic disease that is caused by the obligate intracellular parasitic *Toxoplasma gondii*. This protozoon of the Apicomplexa phyla presents only felines as the definitive host, being the ones where the parasite can complete its life cycle. However, all warm-blooded animals, including mammals and birds, can act as intermediate hosts (**Figure 1**). In most hosts, *T. gondii* causes a lifelong latent infection in tissues such as skeletal and heart muscle, and the central nervous system, causing the disease. In humans, infection by *T. gondii* is particularly important in pregnant women and immunocompromized people. During pregnancy, the risk of fetal infection increases with gestational stage, increasing as gestation progresses [1]. Neonatal manifestations include hydrocephalus, microcephalus, intracranial calcifications, chorioretinitis, cataracts, convulsions, nystagmus, jaundice, petechiae, anemia, enlarged liver and spleen, prematurity, and severe intrauterine growth restriction [2][3]. Ocular manifestations also appear as chorioretinitis and retinal lesions [4]. In immunocompromized people, the neurological symptoms, such as encephalopathy, meningoencephalitis, cerebral mass lesions, headache, confusion, poor coordination, and seizures are usual [5], with toxoplasmic encephalitis being the most frequent manifestation in HIV patients [6], whereas the disseminated toxoplasmosis is more characteristic of transplant patients [7]. However, not only pregnant women and immunocompromized people may suffer the symptoms of *Toxoplasma* infection. Immunocompetent individuals can develop acute, chronic, and ocular toxoplasmosis. The acute toxoplasmosis is asymptomatic around 80% of individuals [8], and the symptoms in the other 20% includes fever, mononucleosis-like symptoms, with cervical posterior adenopathy, myalgia, and asthenia [9]. Although these symptoms are not relatively serious, the severity of infection depends on genotype of the parasite strain. In fact, infections with a highly virulent strain can produce fatal pneumonitis, myocarditis, meningo-encephalitis, and polymyositis [6]. In chronic toxoplasmosis, tachyzoites form bradyzoite cysts intraneuronal which are controlled but not eliminated by the immune system [10]. The immune response in the brain of patients produces brain inflammation, ventricular dilatation, disrupting neuronal structure and connectivity [11][12]. Although the symptoms of chronic toxoplasmosis have not been unraveled, several studies correlated these manifestations with neuropathies [13][14]. Related to ocular toxoplasmosis, it is the primary cause of infectious uveitis, presenting with retinochoroiditis [15].

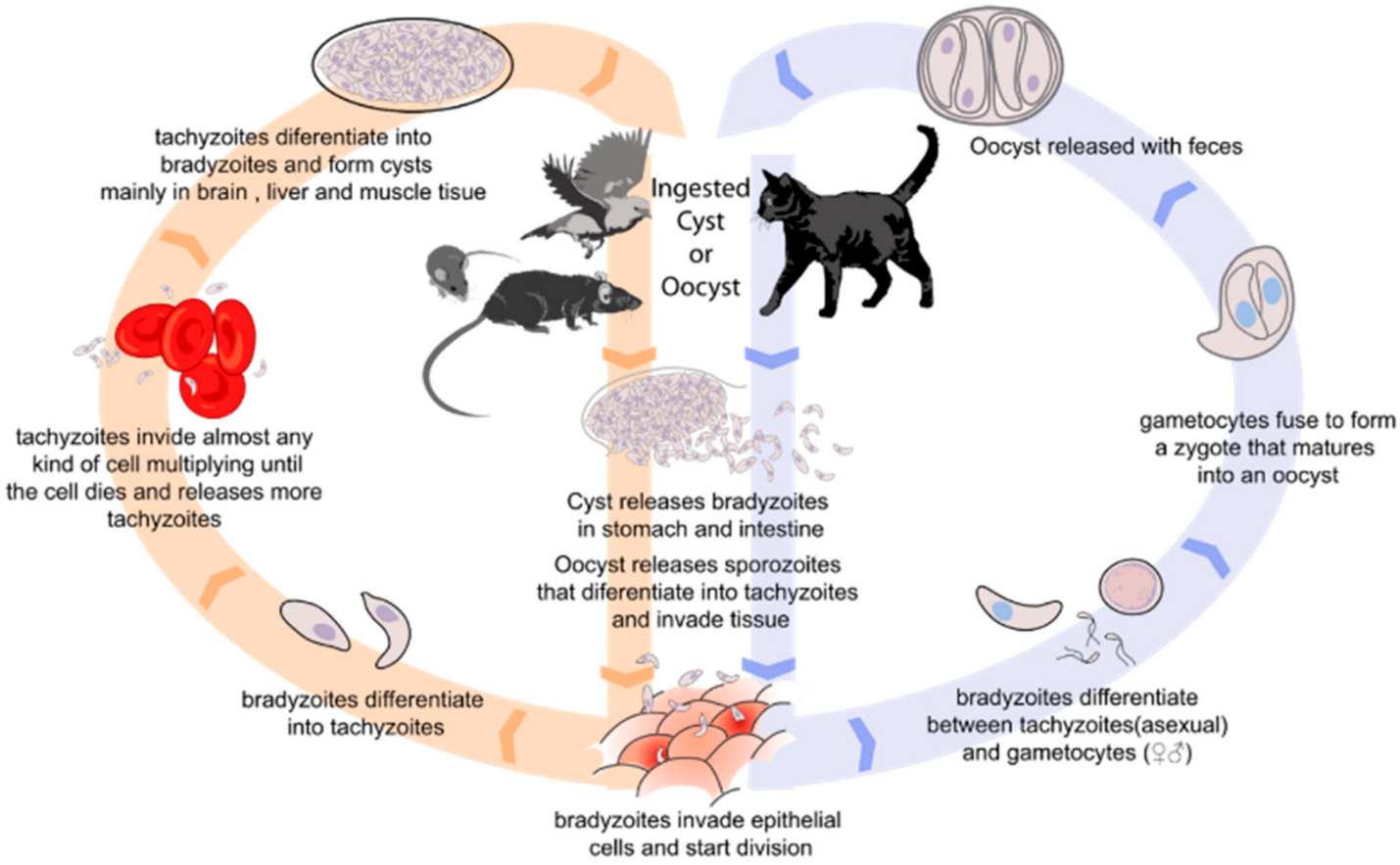

Figure 1. Biological cycle of *Toxoplasma gondii*.

T. gondii has a worldwide geographic distribution and an estimated 30% of the population is seropositive [16]. The genetic diversity of *T. gondii* around the world has been elevated, so more than 36 genotypes have been found [17]. The transmission of this parasite in humans may result from the ingestion of tissue cysts in raw or undercooked meat of infected animals, ingestion of raw vegetables, water that is contaminated with *T. gondii* oocysts from cat feces, and by vertical or transplacental transmission [18]. Although, the main route of infection in humans is through ingestion of contaminated food. In fact, it has been described that up to 50% of infections are caused by food transmission using a novel multiplex Polymerase Chain reaction (PCR) assay [19]. A study that was undertaken in school dining rooms of Colombia showed the presence of *T. gondii* in meat, water, cucumber, and guava juice, both inert and living surfaces [20]. In the last years, the concern about this zoonosis and its transmission has been increasing. In 2018, the EFSA recommended a serological screening of livestock to identify positive farms [21]. In the following year, the EFSA report found that food-borne transmission accounts for 40–60% of *T. gondii* infections [22]. The last report indicated positive samples of meat, fish, raw mollusks and shellfish, honey, and potable water, and *Toxoplasma* was included in category III of zoonotic agents to monitor, along with *Campylobacter* or *Yersinia* [23].

However, and despite the great health public problem that it poses, there are currently no specific detection criteria for *T. gondii* in food, and there are no standardized methods or validation procedures for its detection in the food industry. In fact, different direct and indirect detection techniques exist. Cat and mouse bioassays are the reference

direct techniques to analyze the viability of the parasite, but these test are not commonly used due to the long time that is taken to obtain results, ethical issues, and great costs [18]. The alternative method are cell cultures which are limited in use because of the variability of the results depending the sample [24]. Other serological methods (indirect detection) have been developed such as immunofluorescent assay (IFAT), enzyme-linked immunosorbent assay (ELISA), latex agglutination tests (LAT), modified agglutination test (MAT), and more recently, a luciferase-linked antibody capture assay (LACA) [23][25]. The latest studies of *T. gondii* detection in food products have used serological techniques to improve the sensibility of these serological tests using different approaches. For example, Suwan et al. (2022) used a recombinant dense granule antigen 7 protein for the detection of parasites in blood samples [26]. In addition to these serological methods, other molecular techniques have been tested. Some protocols of PCR have been described as nested PCR, real-time PCR, loop-mediated isothermal amplification (LAMP), and others. However, the more sensitive and specific diagnostic tools to detect *T. gondii* are necessary [27], and the studies about their sensitivity and to unify the detection in different food products are essential to control of parasite infection by food consumption.

2. Methods for *T. gondii* Detection in Food Products

Although *T. gondii* is a high priority foodborne zoonotic pathogen around the world, it is not systematically controlled [28]. At present, there are no specific regulations or ISO standards for the detection of *T. gondii* in any food matrix [21]. Even so, different methods are available to detect tachyzoites, tissue cysts, and oocysts in food products, including immunological and microscopical methods. These methods have an isolate and concentration stage, later applying direct detection methods to the sample. Molecular assays are used to detect the presence of *T. gondii* DNA in samples, while information on the viability and infectivity can be obtained by in vivo assays (usually in mice) or by in vitro culture techniques. A summary of these methods with sensitivity and type of food product where these methods have been used are shown in **Table 1**.

Table 1. The table shows different methods for *T. gondii* detection, sensitivity of method, and type of food product where this method has been used.

Detection Method	Specific Method [1]	Type of Food Product	Detection Range (Sensitivity) [2]	References
Animal model bioassay	Cat	Milk	25%	[29][30]
		Meat	100%	[31]
	Mouse	Milk	100%	[29]

Detection Method	Specific Method [1]	Type of Food Product	Detection Range (Sensitivity) [2]	References
		Meat	100% (10 tachyzoites)	[24]
		Fresh products	13%	[32]
		Bivalve mollusks	2.5%	[33]
		Water	100%	[34]
Cell culture		Meat	100% (10,000 tachyzoites)	[24]
		Milk	-	[30]
Microscopic method		Meat	-	[31]
Molecular methods	PCR	Meat	47.1%	[35]
		Fresh products	95–100%	[36][37]
		Water	100%	[36]
		Milk	100%	[29][38]
		Cheese	100%	[29]
	qPCR	Meat	92.3% (limit 0.01 pg)	[39][40]

Detection Method	Specific Method [1]	Type of Food Product	Detection Range (Sensitivity) [2]	References
		Fresh products	100% (1 oocyst)	[41][42][43]
		Bivalve mollusks	100%	[44]
		Water	100%	[44]
	LAMP	Lymph nodes	85.7%	[45]
		Mussels	5 oocyst/g	[46]
		Fresh products	25 oocyst/50 g	[47]
		Water	100% (1 fg)	[48][49]
Serological methods	IHA	Meat Juice	100% (10,000 oocysts)	[50]
	IFAT	Meat	97%	[51]
		Meat Juice	96.9% (10,000 oocysts)	[50]
	MAT	Meat	86.6%	[51]
		Milk	-	[52]
	ELISA	Milk	-	[30]

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Detection Method	Specific Method [1]	Type of Food Product	Detection Range (Sensitivity) [2]	References
		Meat	91%	[51]
		Meat Juice	100% (10,000 oocysts)	[50]
	BBMA	Meat	98.5%	[53]

[1] PCR: Polymerase chain reaction; qPCR: real-time PCR; LAMP: Loop-mediated isothermal amplification; IHA: indirect hemagglutination antibody; IFAT: indirect fluorescent antibody test; MAT: modified agglutination test; ELISA: Enzyme-Linked Immunosorbent Assay; BBMA: bead-based multiplex assay. [2] The column shows the percentage of samples that were positively detected by the method and the quantity of parasites per quantity of food product that was detected if this data is known. The value (-) means that this data is not known.

3. Prevalence of *Toxoplasma gondii* in Food Products

Transmission through food is the main system of transmission of *T. gondii* to humans [18]. Tissues cysts and tachyzoites are responsible for infection thought meat and milk, respectively [54], and sporulated oocysts can contaminate fresh products, shellfish, and water, and infect humans after consumption [55].

3.1. Meat and Meat Products

T. gondii infections have been reported in all meat production animals around the world, although the prevalence depends on the detection method that is used (**Table 1**). Tissue cysts of parasites in meat are an important source of human infection, due to the fact of that these animals are secondary hosts of the parasite, which can survive long periods of time in these asymptomatic animals, which will later become meat products.

Different techniques are available to detect its presence. The mouse bioassay and PCR are the most widely used direct detection methods, followed by microscopy and the cat bioassay [31]. On the other hand, the MAT, IFAT, and ELISA tests are the most widely used serological methods for the detection of *T. gondii* infection in cattle and meat products [56]. **Table 2** shows the animal and sample that was contaminated, the country of contamination, the method that was used for detection, and prevalence that was found.

Table 2. *T. gondii* in animals and animal products. The table shows the producer animal, sample contaminated, country of contamination, method that was used for detection, and prevalence that was found.

Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
Sheep	Serum	ELISA	150	26 (17.3%)	Iran	[57]
	Serum	ELISA	550	59 (10.8%)	Iran	[58]
	Serum	ELISA	1039	179 (17.2%)	Latvia	[59]
	Serum	MAT	100	42 (42%)	Lebanon	[60]
	Serum	ELISA	64	30 (47%)	Slovakia	[61]
	Serum	DAT	252	148 (58.2%)	Ethiopia	[62]
	Liver	PCR	150	26 (17.3%)	Iran	[57]
	Liver	PCR	90	13 (14.4%)	Iran	[63]
	Heart	PCR	150	48 (32%)	Iran	[57]
	Brain and heart	MAT	136	10 (7.4%)	India	[64]
	Meat juice	ELISA	227	126 (28.6%)	Italy	[65]
	Meat juice	MAT	166	11 (6.6%)	China	[66]
	Meat	PCR	150	33 (22%)	Iran	[57]

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Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
	Meat	PCR	438	43 (9.8)	China	[67]
	Meat	PCR	150	50 (33.3)	Tunisia	[68]
	Meat	ELISA	109	38 (34.9%)	Malaysia	[69]
	Meat	PCR	79	34 (43%)	Australia	[70]
	Meat	PCR	177	3 (1.7%)	India	[71]
Goat	Serum	ELISA	150	16 (10.7%)	Iran	[57]
	Serum	ELISA	185	37 (20%)	Iran	[58]
	Serum	ELISA	445	189 (42.5%)	India	[72]
	Serum	MAT	80	27 (34%)	Lebanon	[61]
	Serum	ELISA	39	8 (21%)	Slovakia	[61]
	Serum	LAT	116	64 (55.2%)	Ethiopia	[62]
	Liver	PCR	150	24 (16%)	Iran	[57]
	Liver	PCR	90	8 (8.8%)	India	[63]

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Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
	Heart	PCR	150	36 (24%)	Iran	[57]
	Brain and heart	MAT	57	4 (7%)	India	[64]
	Meat juice	ELISA	51	14 (27.5%)	Italy	[65]
	Meat	PCR	150	26 (17.3%)	Iran	[57]
	Meat	PCR	254	27 (10.7)	China	[67]
	Meat	PCR	120	39 (32.5)	Tunisia	[68]
	Meat	ELISA	75	41 (54.7%)	Malaysia	[69]
	Meat	PCR	223	3 (1.3%)	India	[72]
Cattle	Serum	ELISA	57	13 (22.8%)	Italy	[73]
	Serum	DAT	2411	313 (13%)	Poland	[74]
	Serum	ELISA	400	52 (13%)	Iran	[75]
	Serum	IFAT	500	2.3 (40.6%)	Brazil	[76]
	Meat	PCR	150	29 (19.3)	Tunisia	[68]

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Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
	Meat	ELISA	392	98 (25%)	Malaysia	[69]
	Meat	PCR	48	5 (10.4%)	Brazil	[76]
Pig	Serum	ELISA	653	4 (0.6%)	Finland	[77]
	Serum	ELISA	447	73 (16.3%)	Denmark	[78]
	Serum	DAT	3111	370 (11.9%)	Poland	[74]
	Serum	IFAT	94	44 (46.8%)	Romania	[79]
	Serum	ELISA	420	56 (23.3%)	Cuba	[80]
	Serum	ELISA	370	14 (3.8%)	Italy	[81]
	Serum	ELISA and IFAT	127	56 (44.1%)	Italy	[82]
	Serum	MAT	375	8 (2.1%)	Italy	[83]
	Serum	ELISA	414	214 (51.7%)	Italy	[84]
	Serum	MAT	182	31 (17%)	Serbia	[85]
	Serum	MAT and IFAT	356	25 (7%) and 48 (13.5%), respectively	Brazil	[86]

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Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
	Serum	MAT and IFAT	400	26 (6.5%)	Brazil	[87]
	Serum	IFAT	60	44 (77%)	Brazil	[88]
	Serum	IHA	784	156 (19.9%)	China	[89]
	Tongue	PCR	60	20 (33.3%)	Brazil	[88]
	Tongue and muscle	PCR	810	54 (6.7%)	India	[90]
	Brain	PCR	339	34 (10%)	China	[91]
	Brain	PCR	107	51 (47.7%)	Italy	[84]
	Heart	PCR	94	25 (26.6%)	Romania	[79]
	Heart	qPCR	103	12 (11.6%)	Italy	[92]
	Diaphragm	PCR	45	15 (33.3%)	Serbia	[85]
	Diaphragm	PCR	1223	107 (8.7%)	China	[93]
	Diaphragm	PCR	60	24 (40%)	Brazil	[88]
	Diaphragm	qPCR	103	2 (1.9%)	Italy	[94]

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Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
	Tissue of seropositive animals	Mouse bioassay	26	18 (69.2%)	Brazil	[87]
	Muscle	PCR	60	23 (38.3%)	Brazil	[88]
	Meat juice	ELISA	212	33 (15.6%)	Denmark	[78]
	Meat	qPCR	118	46 (39%)	Brazil	[94]
	Meat	PCR	498	165 (33.1%)	Italy	[95]
	Meat	PCR	49	3 (6.1%)	Brazil	[76]
	Raw meat products	PCR	3223	175 (5.4%)	Poland	[96]
Chicken	Serum	IFAT	200	72 (36%)	Brazil	[97]
	Serum	ELISA	522	34 (6.5%)	India	[98]
	Serum	LACA	267	29 (10.9%)	Japan	[99]
	Brain	Mouse Bioassay	14	2 (14.3%)	Brazil	[97]
	Heart juice	MAT	1185	230 (19.4%)	USA	[100]

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Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
	Muscle and heart	PCR	522	12 (2.3%)	India	[98]
	Meat	PCR	257	21 (8.2%)	China	[101]
Ducks	Meat	PCR	115	9 (7.8%)	China	[101]
Geese	Meat	PCR	42	2 (4.8%)	China	[101]
Rabbit	Brain and heart	PCR	470	13 (2.8%)	China	[102]
Kibbeh	Meat	PCR	44	1 (2.3%)	Brazil	[76]
Water Buffalo	Serum	MAT and ELISA	197	16 (8.1%) and 13 (6.6%), respectively	Romania	[103]
Ostriches (farmed)	Serum	LAT	409	149 (36%)	Czech Republic	[104]
Common quails (farmed)	Serum	MAT	620	59 (9.5%)	China	[105]
Donkey (farmed)	Meat	PCR	618	57 (9.2%)	China	[106]
Tolai hares (farmed)	Serum	PCR	358	29 (8.1%)	China	[107]

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Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
	Brain	PCR	358	23 (6.4%)	China	[107]
Feral swine	Serum	ELISA	376	34 (9%)	USA	[108]
Wild boar (farmed)	Serum	LAT	882	88 (10%)	China	[109]
Wild boar	Serum	ELISA	331	164 (49%)	Italy	[110]
	Serum	ELISA	181	17 (9%)	Finland	[111]
	Serum	IFAT	26	20 (76.9%)	Brazil	[112]
	Serum	ELISA	306	61 (20%)	Germany	[113]
	Tissue	Mouse bioassay	22	1 (4.5%)	Brazil	[112]
	Brain	qPCR	141	44 (31.2%)	Italy	[114]
	Brain	PCR	263	58 (22%)	Italy	[115]
	Heart	qPCR	166	47 (28.3%)	Italy	[114]
	Heart	PCR	310	70 (22.6%)	Italy	[115]
	Muscle	qPCR	165	40 (24.2%)	Italy	[114]

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Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
	Muscle	PCR	311	74 (23.8%)	Italy	[115]
	Meat juice	ELISA	97	42 (43.3%)	Italy	[116]
	Meat	qPCR	306	37 (12%)	Germany	[113]
Venison	Serum	MAT	914	329 (36%)	USA	[117]
	Heart	Mouse bioassay	36	11 (30.6%)	USA	[117]
Roe deer	Serum	LAT	356	141 (39.6%)	Spain	[118]
	Serum	ELISA	323	130 (40.2%)	Italy	[119]
	Serum	ELISA	184	20 (11%)	Germany	[113]
	Meat	qPCR	184	11 (6%)	Germany	[113]
Fallow deer	Serum	LAT	372	138 (37.1%)	Spain	[120]
	Serum	ELISA	167	17 (10%)	Slovakia	[61]
	Meat	qPCR	80	2 (2%)	Germany	[113]
Red deer	Serum	LAT	553	92 (16.6%)	Spain	[118]

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Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
	Serum	ELISA	96	19 (19.8%)	Italy	[110]
	Serum	ELISA	65	4 (6%)	Germany	[113]
	Meat	qPCR	65	2 (2%)	Germany	[113]
Southern chamois	Serum	LAT	186	26 (14%)	Spain	[118]
Mouflon	Serum	LAT	209	24 (11.5%)	Spain	[118]
	Serum	ELISA	50	12 (24%)	Italy	[110]
Iberian wild goat	Serum	LAT	346	27 (7.8%)	Spain	[118]
Chamois	Serum	ELISA	104	4 (3.8%)	Italy	[110]
Barbary sheep	Serum	LAT	18	1 (5.6%)	Spain	[118]
Moose	Serum	DAT	463	111 (23.9%)	Estonia	[119]
Wild ducks	Brain	qPCR	280	7 (2.5%)	Czech Republic	[120]
	Heart	qPCR	280	11 (3.9%)	Czech	[120]

Sheep Milk and Blood Samples in Relation to Phase of Infection. Vet. Parasitol. 2015, 208, 250–253.

Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
					Republic	
	Muscle	qPCR	280	4 (1.4%)	Czech Republic	[120]
Common pheasants	Brain	qPCR	350	8 (2.3%)	Czech Republic	[120]
	Heart	qPCR	350	4 (1.1%)	Czech Republic	[120]
	Muscle	qPCR	350	3 (0.9%)	Czech Republic	[120]

[1] ELISA: Enzyme-Linked Immunosorbent Assay; MAT: modified agglutination test; DAT: direct agglutination test; PCR: Polymerase chain reaction; LAT: latex agglutination test; IFAT: indirect fluorescent antibody test; qPCR: real-time PCR.

3.2. Milk and Dairy Products

Tachyzoites can be shed in the milk of acutely infected animals, so both raw milk and raw dairy products can pose a risk of infection for consumers [120] (**Table 3**). In fact, one of the factors that is related to infection in the USA is the ingestion of unpasteurized goat's milk [122]. Different studies show the presence of *T. gondii* in milk samples from sheep, goat, camels, and donkeys [52][123][124][125][126], where the prevalence can reach up to 43–65% [127]. However, these data differ between production procedures, management, and techniques of detection, increasing with deficient biosecurity levels (related to the application of a health management program, vaccination protocols, correct quarantines, protocols for visitors, etc.) [128], and were higher for serological rather than molecular techniques. In goat, molecular techniques revealed the presence in 20.6% of milk samples, whereas the ELISA showed 63.3% [129]. Other studies showed that relationship between the prevalence of *T. gondii* antibodies in the goat serum with a prevalence of *T. gondii* DNA in milk samples [130].

Table 3. *T. gondii* in milk and dairy products. The table shows the producer animal, sample contaminated, country of contamination, method that was used for detection, and prevalence that was found.

Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
Donkey	Milk	ELISA	418	41 (9.2%)	China	[123]
Goat	Milk	ELISA	30	19 (63.3%)	Italy	[129]
	Milk	PCR	60	39 (65%)	Poland	[130]
	Milk	ELISA and qPCR	30	27 (90%) and 1 (3.3%), respectively	Egypt	[131]
	Bulk tank milk	ELISA	100	59 (59%)	Italy	[129]
Sheep	Milk	PCR	58	1 (1.7%)	Mongolia	[124]
	Milk	ELISA and qPCR	30	18 (60%) and 1 (3.3%), respectively	Egypt	[131]
Camel	Milk	PCR	9	8 (88.9%)	Mongolia	[124]
	Milk	ELISA and qPCR	30	1 (3.33%) and 0 (0%), respectively	Egypt	[131]
Cattle	Bulk tank milk	ELISA	149	8 (5.4%)	Iran	[132]

[1] ELISA: Enzyme-Linked Immunosorbent Assay; PCR: Polymerase chain reaction; qPCR: real-time PCR.

3.3. Fresh Products and Vegetables

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Fresh products can act as a source of infection in humans. Oocyst detection in environmental and food samples is difficult due to complications in separating and concentrating oocysts from complex matrices, such as raw vegetables, so there is a lack of optimized laboratory methods for its detection [41]. However, Dumètre and Dardé (2003) have proposed possible methods for the detection of *T. gondii* in water, soil, and food samples (mainly, fruit and vegetables), based on methods that are used for other protozoa [133]. Hohweyer et al. (2016) developed an immunomagnetic separation assay (IMS) targeting the cell wall of oocysts, although it is not yet commercially available [134]. In addition to conventional methods such as microscopy, PCR or qPCR, a LAMP test has been developed to detect *T. gondii* in experimental contaminated baby ready-to-eat lettuces. The detection limit of this method was approximately 25 oocysts per 50 g of lettuce leaves [47]. Recently, special RT-PCR assay has been developed and it was effective to discriminate viable *T. gondii*, detecting two to nine oocysts per gram of spinach [43].

The first detection of *T. gondii* DNA in fruits and vegetables was in 2012 [135]. Nowadays, some studies have linked acute outbreaks of human toxoplasmosis with the ingestion of oocysts, where green vegetables have been identified as a possible vehicle of infection which can be contaminated by irrigation water [136][137][138]. In fact, Pinto-Ferreira et al. (2019) undertook a meta-analysis and concluded that vegetables will be the most common possible route of transmission in the future [139]. Contamination by *T. gondii* has been observed in different vegetables around the word, including lettuce, chicory, rocket, parsley, spinach, pack choi, cabbage, rape, asparagus, endive, Chinese chives, carrots, cucumbers, strawberries, and radish [140] (**Table 4**).

Table 4. *T. gondii* in fresh products and vegetables. The table shows the product that was analyzed, country of contamination, method used for detection, and prevalence that was found.

Product Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
Mixed-salad packages	qPCR	648 packages	5 (0.8%)	Italy	[141]
	PCR	90 packages	8 (8.9%)	Czech Republic	[142]
Leafy greens	qPCR	152	45 (29.6%)	Morocco	[143]
Carrot	qPCR	30	3 (10%)	Morocco	[144]
	qPCR	46	9 (19.5%)	Poland	[135]

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Product Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
	PCR	93	7 (7.5%)	Czech Republic	[142]
Chicory	PCR	40	2 (5%)	Brazil	[145]
Red cabbage	qPCR	8	1 (1.2%)	China	[42]
Coriander	qPCR	29	8 (27.6%)	Morocco	[144]
Cucumber	PCR	109	13 (11.9%)	Czech Republic	[142]
Lettuce	qPCR	28	3 (10.7%)	Morocco	[144]
	qPCR	50	9 (18%)	Poland	[135]
	qPCR	71	5 (7%)	China	[42]
	PCR	168	5 (3%)	Brazil	[145]
Spinach	qPCR	50	2 (4%)	China	[42]
Parsley	qPCR	29	13 (44.8%)	Morocco	[144]
	PCR	5	1 (20%)	Brazil	[145]
Pak Choi	qPCR	34	1 (2.9%)	China	[42]

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Product Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
Radish	qPCR	16	1 (6.3%)	Morocco	[144]
	qPCR	60	3 (5%)	Poland	[42]
Rape	qPCR	22	1 (4.5%)	China	[42]
Rocket	PCR	7	1 (14.3%)	Brazil	[145]

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[1] PCR: Polymerase chain reaction; qPCR: real-time PCR.

3.4. Marine Products

Aquatic environments can be contaminated with wastewater carrying *T. gondii* oocysts. Mollusks such as clams, mussels, oysters, and scallops, filter-feed and trap phytoplankton in the gills. This filter feeding process can also concentrate waterborne pathogens within their tissues, including oocysts, which can survive for long periods of time in both fresh- and salt-water [146]. For detection in mollusks, samples of whole tissue or organs can be used and the most frequent techniques that are used are those that are based on PCR, generally directed to the B1 gene [147][148]. Various molecular methods have been used for detection in fish, such as PCR, qPCR, and RT-PCR, targeting the same gene, or the 529 bp DNA repeat element. The last method seems more sensitive, with the five oocysts as a low limit of detection. But it is no more specific, requiring direct sequencing for definitive confirmation of *T. gondii* [149]. In addition, the techniques have been carried out in different matrices, such as the digestive tract, muscle, brain, and even gills, among others [150]. Serological techniques have also been used for the detection in fish, such as ELISA, by detecting IgG and IgM, suggesting the fish are actually infected with *T. gondii* [151], rather than just serving as paratenic hosts such as shellfish.

The consumption of raw mollusks is considered a risk factor for *T. gondii* infection. **Table 5** shows the prevalence of parasite in different mollusks, bivalves, and fishes.

Table 5. *T. gondii* in marine products. The table shows the animal, sample contaminated, country of contamination, method that was used for detection, and prevalence that was found.

Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
Bivalve shellfish	Tissue	PCR	2907	82 (2.8%)	China	[152]
Green-lipped mussels	Tissue	PCR	104	13 (16.4%)	New Zealand	[153]
Mediterranean mussel	Gills	qPCR	53 pools at 795 specimens	21 (39.6%)	Turkey	[147]
Clam	Tissue	qPCR	61 pools at 1020 specimens	4 (6.6%)	Tunisia	[148]
	Digestive gland	PCR	390	6 (1.5%)	Canada	[154]
	Haemolymph	PCR	390	2 (0.6%)	Canada	[154]
Mediterranean scald fish	Gills	PCR	1 pool at 6 specimens	1 (100%)	Italy	[155]
Pacific oyster	Gills	PCR	6 pools at 109 specimens	1 (16.67%)	Italy	[156]
Oyster	Mantle, gills, and rectum	qPCR	1440	447 (31%)	USA	[157]
Bogue	Gills	PCR	26 pools at 260 specimens	4 (15.4%)	Italy	[155]

Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
	Intestine	PCR	26 pools at 260 specimens	3 (11.5%)	Italy	[155]
	Muscle	PCR	26 pools at 260 fish	6 (23.1%)	Italy	[155]
White seabream	Muscle	PCR	3 pools of 18 specimens	1 (33.3%)	Italy	[155]
European anchovy	Gills	PCR	35 pools at 350 specimens	2 (5.7%)	Italy	[155]
	Intestine	PCR	35 pools at 350 specimens	1 (2.9%)	Italy	[155]
European hake	Gills	PCR	15 pools at 90 specimens	1 (6.7%)	Italy	[155]
	Muscle	PCR	15 pools at 90 specimens	1 (6.7%)	Italy	[155]
Red mullet	Intestine	PCR	11 pools at 110 specimens	3 (27.3%)	Italy	[155]
American prawn	Muscle	PCR	618	4	China	[155]
Nippon shrimp	Muscle	PCR	813	1	China	[158]

Animal	Sample Analyzed	Detection Method [1]	Number of Samples Tested	Number of Positive Samples (%)	Location	Reference
Axillary seabream	Gills	PCR	8 pools at 80 specimens	2 (25%)	Italy	[155]
	Intestine	PCR	8 pools at 80 specimens	1 (12.5%)	Italy	[155]
	Muscle	PCR	8 pools at 80 specimens	1 (12.5%)	Italy	[155]
Common pandora	Gills	PCR	3 pools at 18 specimens	1 (33.3%)	Italy	[155]
	Intestine	PCR	3 pools at 18 specimens	2 (66.7%)	Italy	[155]
	Muscle	PCR	3 pools at 18 specimens	1 (33.3%)	Italy	[155]
Thornback ray	Muscle	PCR	1 fish	1 (100%)	Italy	[156]
Red scorpionfish	Intestine	PCR	1 pool at 3 specimens	1 (100%)	Italy	[155]
Blotched picarel	Muscle	PCR	4 pools at 24 specimens	1 (25%)	Italy	[155]
Atlantic horse mackerel	Muscle	PCR	15 pools at 120 specimens	4 (26.7%)	Italy	[155]

[1] PCR: Polymerase chain reaction; qPCR: real-time PCR.

4. Control and Food Safety

The control of *T. gondii* infection must be done at several levels. First, certain risk factors increase the prevalence of the parasite in farm animals. Hygienic management practices and correct management which involves keeping cats away from crops and gardens and animal feed, are essential to control this pathogen in farms [89]. Temperature and humidity control could decrease the survival and distribution of the parasite, as well as a late replacement of the animals, since older animals present higher prevalence than young ones [62][159]. The intensive systems of production present lower prevalence than extensive or semi-intensive ones [128]. In the same way, organic farms present higher prevalence than conventional farms, probably due to due to the high risk of being exposed and infected with environmental oocysts of parasites or from ingested infected rodents [78]. Nevertheless, the most important factor in all production systems seems to be the biosecurity level (control of exposition and infection of animals with environmental parasites and control of domestic animals that are infected near the farms) and early detection [78][80]. Consumption of fresh milk and dairy products are other of factors that cause *T. gondii* infection in humans. In fact, pasteurization of milk and milk products is also an important control measure. Undoubtedly, stopping consuming these types of products could considerably reduce the prevalence of infection in humans. On the other hand, as occurs in meat products, adequate hygienic and sanitary conditions on farms would lead to this reduction. In fresh products and vegetables, the most common mechanism of contamination is irrigation with water that is contaminated by oocysts, so sanitary control measures in irrigation water would be interesting. Furthermore, washing fresh produce after harvest and before consumption is an important control measure, since the chemical disinfectants are not effective [18].

The control of *T. gondii* in food production is essential. However, control measures during food inspection are not applied [21]. Currently, different methods of inactivation exist, although in the industry they are not applied directly for the control of this parasite. The most used methods of control are thermal methods, including both high and low temperatures. Heat treatments can destroy oocysts from both sporulated and non-sporulated strains. It is also possible to eliminate bradyzoites and tachyzoites, although the elimination of the first requires higher temperatures and longer times [160][161]. Relationship between raw meat or other animal products have been demonstrated by several studies. In meat products, the main control measure to prevent infection is an adequate cooking and proper prevention of cross-contamination [162]. In fact, *T. gondii* can be eliminated from meat in 5–6 min at 49 °C, in 44 s at 55 °C, or in 6 s at 61 °C [163]. Different meat products require different temperature conditions for inactivation. For example, beef should be cooked at least 63 °C; whereas pork meat, minced meat, and bushmeat at 71 °C; and poultry at 82 °C. In general, meat should be cooked to at least 67 °C before consumption. In dairy products, the pasteurization of milk, at 63 °C for 30 min is sufficient to eliminate tachyzoites [164]. Rani and Pradhan (2021) published an exhaustive study that was related to the survival of *T. gondii* during cooking and low temperature storage and concluded that the parasite was not found when the internal temperature reached 64 °C and below −18 °C [165].

However, these elevated temperatures are not applicable to all food matrices. This is the case of vegetables and fresh products [166]. Regarding inactivation by low temperatures, it has been shown that freezing can inactivate tissue cysts of *T. gondii*. To inactivate isolate tissue cysts, a minimum of three days is required at −20 ºC [167]. In addition to thermal methods, other non-thermal methods can be used for the inactivation, such as high-pressure processing [146][168][169], ionizing radiation [170][171], and curing or salt [34][172]. The inactivation of *T. gondii* in food for thermal and non-thermal methods has been extensively analyzed in the review that was published by Mirza et al. (2018) [173].

The inactivation of *T. gondii* in food products has been realized traditionally with high temperatures (thermal methods) and when cured and salted [165][174], whereas the non-thermal methods are presented as emerging technologies for the control of *T. gondii* in food. High pressure processing (HHP) is a novel method for liquid and solid food products where pressures of 340–550 MPa during 1 min can inactive cysts of the parasite [175]. The second new method is ionizing radiation (IR), which is capable of inactivating or killing *T. gondii* cysts in meat [160]. However, these methods have not yet been tested in other food matrices or to inactive other parasitic forms.