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Toxoplasma gondii in Spanish commercial dry-cured meat products

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ABSTRACT

Toxoplasmosis is an infection caused by *Toxoplasma gondii*, the transmission of which has usually been attributed to ingestion of undercooked or raw meat. Epidemiological studies also mention cured meat products as a potential risk factor for acquiring toxoplasmosis. With the aim of contributing to the risk assessment process, 552 samples of commercial dry-cured hams/shoulders and dry-cured sausages of different trademarks from different localities in Spain were randomly purchased for analysis. These were, specifically, 311 dry-cured hams/shoulders and 241 dry-cured sausages (76 samples of *chorizo*, 75 samples of *fuet/longaniza*, and 90 samples of *salchichón*). Additionally, data featured on labels of each meat product were gathered with the purpose of studying the influence of curing time and salt content, among other parameters, on the viability of *Toxoplasma*. Real-time polymerase chain reaction technique (qPCR) was performed to detect *T. gondii* DNA in the samples, and infectivity was determined by mouse bioassay of positive qPCR samples. The presence of *T. gondii* was detected in 57 samples (10.3%), with a parasite load between 28.05 and 35.66 Ct. Bioassay test showed that 47 out of the 57 meat products in which the parasite has been detected produced mice seropositive response (IFA), which represents 8.5 of the total number of samples analyzed. Of those samples, DNA of *Toxoplasma gondii* in mice brain was detected in 6 meat products, indicating its viability (1.1%). Ct values obtained by qPCR in the brains of seropositive mice ranged from 33.10 to 36.04. According to product type, the parasite was viable in 3 dry-cured ham/shoulder samples and in 3 *salchichón* samples. Statistical analysis showed that none of the variables under consideration detailed on the meat product labels had a significant influence on the viability of the parasite. In conclusion, we found a low prevalence of the infective forms of *Toxoplasma gondii* in cured meat products, although the risk for consumers cannot be completely excluded. However, scientific monitoring of commercial meat products continues to be necessary in order to provide data for risk assessment of *Toxoplasma gondii* through the meat industry's Hazard Analysis and Critical Control Point (HACCP-based self-control system). In order to ensure that consumers can make a safe choice among these ready-to-eat products, it is important for food labels to include information on those parameters which are relevant for the survival of the parasite, such as curing times, or freezing treatment of meat used as an ingredient.

1. Introduction

Toxoplasma gondii, an obligate intracellular protozoan parasite widespread in humans and animals around the globe, is a causative agent of toxoplasmosis (Ybañez et al., 2020). In fact, all warm-blooded animals can act as intermediate hosts, although the life cycle is only completed in the definitive hosts (cats and other felines) (EFSA & ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2021). Infection in humans is mostly non-symptomatic, although in immunocompromised individuals it can be life-threatening (Vismarra et al., 2022).

The European Food Safety Authority (EFSA) identifies *T. gondii* as one of the most relevant parasites among all pathogens listed as the main agents of food-borne diseases, and estimates that food-borne transmission accounts for 40–60% of all *T. gondii* infections (EFSA & ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2021).

Consumption of undercooked or raw meat from farm animals containing viable cysts has been indicated as a major source of *T. gondii* infection (Almeria & Dubey, 2021; Condoleo et al., 2018; EFSA & ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2021; Guo et al., 2015; Juránková et al., 2014;

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Opsteegh et al., 2011). What is more, EFSA names *Toxoplasma* as one of the most relevant biological risks in the context of pork meat inspection. Nevertheless, current regulations do not establish criteria for its detection during meat inspection (EFSA, 2011).

Food processing can provide an additional barrier against *T. gondii*. Many authors have reported curing as an effective technology (Genchi et al., 2017; Hill et al., 2018; Franssen et al., 2019; Gomez-Samblas et al., 2021), although other studies highlight that the curing process may be insufficient to inactivate *T. gondii* bradizoite cysts (Gomez-Samblas et al., 2015; Herrero et al., 2017; Warnekulasuriya et al., 1998). In fact, the parasite's loss of viability in dry-cured meat products is dependent on factors such as curing time, salt content, water activity, pH, and fat content. Actually, some authors consider that dry-cured meat products with high fat content, elaborated with low sodium chloride concentration and cured for a short period of time pose a potential risk for susceptible individuals (Fredericks et al., 2020; Herrero et al., 2017; Pott et al., 2013). Hill et al. (2018) and Fredericks et al. (2019, 2020) have described and updated parameters for inactivation of *T. gondii* using a model curing process that can be easily implemented by the pork industry for a variety of different dry cured RTE products (sausages and hams). This is especially important in view of the fact that dry-cured meats are ready-to-eat (RTE) products, i. e., they can be consumed without prior cooking (Cardoso-Toset et al., 2017; Morales-Partera et al., 2017; Syne et al., 2013).

This is confirmed by epidemiological studies, which have identified the consumption of cured or fermented meat products as a potential risk factor for infection with *T. gondii* (Buffolano et al., 1996; Condoleo et al., 2018; Cook et al., 2000; Jones et al., 2009). Given the possibility of the presence of *T. gondii* in pork meat, the frequent use of pork as a major ingredient in dry-cured meat products enhances the potential risk of consumer infection (Fredericks et al., 2019).

A great variety of dry-cured meat products with varying composition and a wide range of curing times are available on the market. Food information made available to the final consumer by means of a label (or any other means) helps to guarantee consumers' right to have access to information that enables them to use foodstuffs safely. Regulation (EU) No. 1169/2011 establishes the general principles, requirements, and responsibilities governing food information, and, in particular, food labelling. Regarding the above-described parameters that can influence the viability of *T. gondii*, food ingredients and a nutrition declaration are the only mandatory elements, while information regarding curing times is not always provided. However, the Spanish quality standard for cured hams/shoulders obtained from white pig allows for the optional use of the terms *bodega*, *reserva*, or *gran reserva* on the label, which reflect minimum maturation times (Real Decreto 474/2014, 2014). Similarly, the Spanish quality standard for Iberian hams/shoulders establishes a minimum curing period for these products (Real Decreto 4/2014), although other meat cured products are not governed by a similar legal norm.

The aim of the current study was to provide prevalence data of *T. gondii* in Spanish RTE dry-cured meat products (hams/shoulders, *chorizo*, *fuet/longaniza*, and *salchichón*) made commercially available to consumers, as well as to take into account the information featured on the label regarding parameters that can be related to the viability of the parasite. Our results are intended as a contribution to the risk assessment of human toxoplasmosis associated with the consumption of these RTE products. To the best of our knowledge, this is the first study that includes label information in the analysis of experimental research results.

2. Materials and methods

2.1. Sampling of commercial dry-cured meat products and sample description

Sampling was designed and carried out by the VISAVET group (Veterinary School, Universidad Complutense de Madrid, Spain), a partner with our research team in a coordinated research project that includes the current study. In total, 552 samples of Spanish commercial dry-cured meat products of different brands and with varying commercial presentations were randomly purchased in supermarkets and retail stores for analysis. Specifically, 311 dry-cured hams/shoulders and 241 dry-cured sausages (76 samples of *chorizo*, 75 samples of *fuet/longaniza*, and 90 samples of *salchichón*) were included in the study (Table 1). Samples were received at our laboratory and stored in refrigeration until analysis.

Dry-cured ham/shoulder is a product manufactured according to the following basic principles: curing is done with salt and nitrites, and stabilization is achieved through decreased water activity. This procedure does not require heat treatment before consumption. A curing compound consisting of salt and other ingredients, which may include sugar, nitrates, phosphates, and other seasonings, is rubbed on the surface of the ham/shoulder. The ham/shoulder is then hung to dry, allowing it to age over a period ranging from a few weeks to over a year, depending on product type, although the aging process tends to be approximately six months. During that time, the curing compound penetrates the entire piece of meat, drawing out moisture and thereby preserving the ham/shoulder. The meat product's weight is reduced by 18–25%.

Chorizo, *salchichón*, and *fuet/longaniza* are dry-cured sausages usually made with a mixture of comminuted pork, pork fat, salt, sugar, spices, and authorized additives (e.g., nitrites and nitrates, as well as antioxidants), stuffed into natural or artificial casings, and subjected to sufficient drying and maturing to obtain stability along with characteristic color, taste, and flavor. Spices commonly used in the manufacture of *chorizo* are paprika, garlic, black or white pepper, nutmeg, and oregano. The typical ingredients of *salchichón* are whole black pepper, nutmeg, and cilantro. *Longaniza* and *fuet* are of the same composition as *salchichón*, but stuffed into a smaller natural casing (usually less than 40 mm).

Our study was performed on the main types of product available on the market: packaged slices/cubes (n = 436) and entire pieces (n = 116). These were, in particular, 299 samples of packaged slices of dry-cured ham/shoulder, 12 of packaged cubes of dry-cured ham/shoulder, 116 entire dry-cured sausages, and 125 packaged sliced dry-cured sausages.

2.2. Record of meat product labelling information

Data of interest from the mandatory information provided by the label (Regulation (EU) No 1169/2011) were recorded for each sample: name of the meat product, commercial presentation, pig breed, curing time, data included in the nutritional declaration (quantity of fat and salt), spices, and additives (preservatives and antioxidants).

When the curing time of hams/shoulders was not indicated on the label, it was estimated on the basis of the use of specific terms (when available) as regulated by Spanish quality standards (Real Decreto 474/2014 and Real Decreto 4/2014). According to these food laws, the name of the product can include the following terms: *bodega*, *reserva*, or *gran reserva* when the minimum maturation time for hams is 9, 12, or 15 months, respectively (and 5, 7, or 9 months, respectively, for shoulder cuts). A minimum of 600–730 days of curing is required for Iberian hams, and 365 days for shoulder cuts. A similar legal norm does not exist for other meat cured products.

2.3. Analysis of dry-cured meat products

Samples were homogenized and then analyzed by qPCR before the end of their minimum durability date ("Best before" date). Positive samples were subsequently analyzed by mouse bioassay to determine the viability of the parasite.

2.3.1. qPCR analysis of dry-cured meat products

To determine the presence of *T. gondii* DNA, acid pepsin digestion

Table 1
Sample description and label information.

		n	Dry-cured meat product				
			Ham/Shoulder	*Chorizo*	*Fuet/Longaniza*	*Salchichón*	Total
			311	76	75	90	552
Commercial presentation	Entire pieces		–	40.8% (31)	100% (75)	11.1% (10)	**21.0% (116)**
	Packaged slices		96.1% (299)	59.2% (45)	–	88.9% (80)	**76.8% (424)**
	Packaged cubes		3.9% (12)	–	–	–	**2.2% (12)**
Pig breed	White		82.3% (256)	81.6% (62)	94.7% (71)	67.8% (61)	**81.9% (452)**
	Iberian		17.7% (55)	18.4% (14)	2.7% (2)	32.2% (29)	**18.1% (100)**
Time of curing (months)[a]	<5		–	100% (76)	100% (75)	100% (90)	**43.7% (241)**
	5–9		57.9% (180)	–	–	–	**32.6% (180)**
	10–14		16.4% (51)	–	–	–	**9.2% (51)**
	15–19		12.9% (40)	–	–	–	**7.2% (40)**
	>20		11.6% (36)	–	–	–	**6.5% (36)**
Salt content (g/100 g)[b]			4.53 ± 0.93	3.49 ± 0.73	4.17 ± 0.44	3.84 ± 0.53	**4.21 ± 0.88**
Fat content (g/100 g)[b]			16.83 ± 9.48	28.40 ± 12.32	27.89 ± 11.28	24.67 ± 9.94	**21.14 ± 11.38**
Spices	Yes		–	78.9% (60)	56% (42)	57.8% (52)	**27.9% (154)**
	No		100% (311)	21.1% (16)	44% (33)	42.2% (38)	**72.1% (398)**
Preservatives	Yes		93.6% (291)	64.5% (49)	100% (75)	92.2% (83)	**90.2% (498)**
	No		6.4% (20)	35.5% (27)	–	7.8% (7)	**9.8% (54)**
Antioxidants	Yes		64.0% (199)	61.8% (47)	98.7% (74)	92.2% (83)	**73.0% (403)**
	No		36.0% (112)	38.2% (29)	1.3% (1)	7.8% (7)	**27.0% (149)**

[a] When curing time was not indicated on the label, we estimated it on the basis of the use of specific terms (when available) regulated by Spanish quality standards (Real Decreto 474/2014 and Real Decreto 4/2014). Nevertheless, 4 samples did not feature that information.
[b] Mean ± standard deviation.

was applied to 50 g of sample prior to qPCR analysis, as described elsewhere (Bayarri et al., 2010; Dubey, 1998). The sediment was resuspended in 3 ml of distilled water purified for use in PCR; an aliquot of 200 μl was used for DNA extraction and identification of *T. gondii*, carried out as described in Gracia et al. (2020). A portion of each sample was kept refrigerated for bioassay analysis in case of a positive PCR result.

2.3.2. Analysis of dry-cured meat products by mouse bioassay

qPCR positive samples were analyzed by mouse bioassay to determine viability of *T. gondii*. For this purpose, acid pepsin digestion (Bayarri et al., 2010; Dubey, 1998) was applied to 50 g of sample and a 0.5 ml aliquot of digestion extract was inoculated intraperitoneally into each of eight 20–25 g CD1 Swiss female mice per sample (Janvier Labs, Le Genest-Saint-Isle, France). If the parasite was viable in the cured-meat sample, tissue cysts could form in the brain of exposed mice and tissue cyst formation could be confirmed by qPCR. All experiments included negative control mice. The mice were certified pathogen-free and kept at the Centro de Investigación Biomédica de Aragón (CIBA) in Zaragoza (Spain). Inoculation, maintenance, and euthanasia of the mice were performed under the standards of the Ethics Advisory Commission for Animal Experimentation and the Biosecurity Commission of the University of Zaragoza, as granted by Judgment No PI55/14. These guidelines are in accordance with the Protocol of International Guiding Principles for Biomedical Research Involving Animals (Directive 2010/63/EU).

2.3.2.1. IFA of mouse sera. IFA of mouse sera was carried out for screening purposes of potential viability. Blood samples were drawn from mice that survived 60 days after inoculation. Sera samples of mice were analyzed by IFA to detect antibodies against *T. gondii* as described by Herrero et al. (2017).

2.3.2.2. DNA extraction and identification of T. gondii in mice brains. Analysis of *T. gondii* DNA from brains of serologically positive mice was performed by qPCR to determine viability of the parasite. A piece of 15 mg from each brain was homogenized through hand pistel plastic rotating plungers in an Eppendorf tube; analysis was performed as described by Gracia et al. (2020).

The inoculated mice were considered infected with *T. gondii* when antibodies to *T. gondii* were demonstrable in their sera and DNA of *Toxoplasma* was found in their brains.

2.4. Statistical analysis

A descriptive statistical analysis of the label information, as well as the presence and viability of *T. gondii* in dry-cured meat products, was performed, and confidence intervals were calculated using Wilson's Score method (Wilson, 1927). The quantitative variables (quantity of fat and salt) were described in terms of their mean and standard deviation.

Additionally, the results for the presence and viability of *Toxoplasma* in the dry-cured meat product samples were statistically related to the labelling information. In the case of qualitative variables (pig breed, commercial presentation, curing time, additives [preservatives and antioxidants] and spices), statistical analyses were performed using Pearson's Chi-square test (or Likelihood Ratio test when the Chi-square test was not valid because more than 20% of expected frequencies were lower than 5).

To study the association between dichotomic and quantitative variables, we first determined the normality of the quantitative variables by applying the Shapiro-Wilk test for all categories of categorical variable. If the variables showed a normal distribution for all categories, we assessed the association with a dichotomic variable using Student's t-test for independent samples; when the normality hypothesis was rejected, we used the Mann-Whitney test as a nonparametric alternative. In case of more than two categories, ANOVA test was used as parametric test, and Kruskal-Wallis test as nonparametric test.

All statistical analyses were performed with the IBM SPSS 19.0 statistical software for Windows. Differences were considered statistically significant when p-value <0.05.

3. Results and discussion

Data gathered from the label information of all analyzed meat products are shown in Table 1. All of them provided the mandatory information required by the Regulation (EU) No. 1169/2011. Out of all the samples, 452 were from white pigs (81.9%) and 100 from Iberian pigs (18.1%). The rang of curing times for ham and shoulder was mainly 5–9 months (180/311, 57.9%) and, to a lesser extent, 10–14 months (51/311, 16.4%), 15–19 months (40/311, 12.9%), and over 20 months

(36/311, 11.6%). Three samples of ham and one shoulder sample lacked curing time information. For all the sausages under analysis, a curing time of less than 5 months was estimated.

Average salt content was 4.39 g salt/100 g in dry-cured ham/shoulder and 3.91 g salt/100 g in sausages. Hams (4.53 g salt/100 g) had salt content comparable to that of shoulders (4.49 g salt/100 g) (Mann-Whitney p-value = 0.431), whereas among sausages the highest salt content was found in *fuet/longaniza* (4.17 g salt/100 g) (Kruskal-Wallis p-value<0.001). As for fat content, the mean value for ham and shoulder was 16.83 g fat/100 g, which was significantly lower (Kruskal-Wallis p-value<0.001) than fat content of sausages (average of 26.85 g fat/100 g with a minimum of 24.67 g fat/100 g in *salchichón* and a maximum of 28.40 g fat/100 g in *chorizo*). Spices were only used as an ingredient in sausage samples (154 out of 241, 63.9%). The spices specified on the labelling were paprika, garlic, nutmeg, pepper, oregano, rosemary, and cayenne. The sausage samples analyzed in this study contained those spices alone or in combination.

The total number of meat products that included preservatives in their formulation was 498 (90.2%). The preservatives used were potassium sorbate (E 202), natamycin (E 235), sodium nitrite (E 250), sodium nitrate (E 251), potassium nitrate (E 252), and lactic acid (E 270), whereby E 250 and E 252 were the preservatives most frequently declared (90.8 and 95.6%, respectively). Antioxidants were used in the formulation of 403 samples (73.0%). The declared antioxidants were: ascorbic acid (E−300), sodium ascorbate (E−301), tocopherol-rich extract (E−306), sodium erythorbate (E−316), sodium lactate (E−325), potassium lactate (E−326), calcium lactate (E−327), sodium citrates (E−331), and extracts of rosemary (E−392). The most prevalent antioxidants were E−301 (68.7%) and E−300 (37.2%).

To the best of our knowledge, published studies on the presence and viability of *T. gondii* in commercial samples of dry-cured meat products are scarce (Bayarri et al., 2012; Costa et al., 2018; Fallah et al., 2011; Gómez-Samblás et al., 2015; Rahdar et al., 2012; Sroka et al., 2019; Warnekulasuriya et al., 1998) and some of them do not focus on pork products, or the meat type is not indicated (Azizi et al., 2014; Fallah et al., 2011; Rahdar et al., 2012). In addition, none of them have evaluated the relationship between labelling information and experimental results. What is more, detailed descriptions of analyzed meat products regarding their composition and/or their technological processes are lacking (Azizi et al., 2014; Fallah et al., 2011; Rahdar et al., 2012; Warnekulasuriya et al., 1998).

We detected *T. gondii* in 57 samples (10.3%; 95%CI: 8.1%, 13.1%), with a parasite load ranging between 28.05 and 35.66 C t (Table 2). Specifically, positivity was detected in 40 of 311 dry-cured ham/shoulder samples (12.9%; 95%CI: 9.6%, 17.0%), 1 of 76 *chorizo* samples (1.3%), 12 of 75 *fuet/longaniza* samples (16.0%; 95%CI: 9.4%, 25.9%), and 4 out of 90 samples of *salchichón* (4.4%; 95%CI: 1.7%, 10.9%). These results differ from those obtained in a previous study carried out in our laboratory, in which the parasite was not detected in 25 samples of dry-cured ham purchased in Spanish retail outlets. Samples included paleta and ham, white and Iberian pig, and packaged slices of different trademarks as well as pieces cut on request (Bayarri et al., 2012).

The only significant difference in terms of product type revealed by statistical analysis (Pearson's Chi-square test, p-value = 0.002) was a greater presence of the parasite in hams/shoulders (12.9%) than in sausages (7.1%). All other variables (commercial presentation, pig breed, curing time, fat and salt content, spices and additives) did not exert a significant influence, although it could be reasonably expected that Iberian dry-cured meat products would exhibit a significantly greater presence of the parasite. In fact, Gómez-Samblás et al. (2021) stated that it is rather difficult to control toxoplasmosis in Iberian pigs, as they are traditionally raised in extensive outdoor farms, where contact with intermediate hosts (cats and rats) is hard to avoid.

Bioassay test showed that 47 out of the 57 meat products in which the parasite has been detected produced mice seropositive response (IFA), which represents 8.5% (95%CI: 6.5%, 11.1%) of the total number

Table 2
Parasite load and viability of *Toxoplasma gondii* in positive dry-cured meat products.

Dry-cured meat product	Parasite load	Viability		
	Meat product extract qPCR[a]	Mouse serum IFA[b]	Mouse brain PCR[c]	*T. gondii* viability
Ham/Shoulder	28.05	1/4	0/1	Negative
	28.05	4/4	0/4	Negative
	28.91	2/4	0/2	Negative
	29.86	3/4	0/3	Negative
	29.91	0/4	–	Negative
	30.51	0/4	–	Negative
	30.56	3/3	1/3 (33.20)	**Positive**
	30.61	0/4	–	Negative
	31.00	2/4	0/2	Negative
	31.52	4/4	0/4	Negative
	31.74	2/4	0/2	Negative
	32.05	2/4	0/2	Negative
	32.08	0/4	–	Negative
	32.18	2/4	0/2	Negative
	33.16	1/2	0/1	Negative
	33.71	2/4	0/2	Negative
	33.78	2/4	0/2	Negative
	33.28	1/3	0/1	Negative
	32.02	1/1	0/1	Negative
	29.97	1/1	0/1	Negative
	35.66	1/4	0/1	Negative
	28.95	1/1	0/1	Negative
	31.32	2/4	0/2	Negative
	31.32	2/4	0/2	Negative
	30.27	0/4	–	Negative
	34.91	2/4	0/2	Negative
	34.92	1/4	0/1	Negative
	35.53	0/4	–	Negative
	35.30	2/4	0/2	Negative
	34.80	3/4	1/3 (34.14)	**Positive**
	35.23	2/4	0/2	Negative
	35.16	1/4	0/1	Negative
	35.34	2/4	0/2	Negative
	32.72	2/4	0/2	Negative
	34.91	1/4	0/1	Negative
	34.57	1/4	0/1	Negative
	34.51	2/4	0/2	Negative
	35.40	1/4	1/1 (35.13)	**Positive**
	33.78	2/4	0/2	Negative
	30.62	0/4	–	Negative
TOTAL OF HAM/SHOULDERS	**40 positive samples**	**3 infective samples**		
Chorizo	30.02	0/4	–	Negative
Fuet/Longaniza	28.34	1/1	0/1	Negative
	29.12	3/4	0/3	Negative
	30.32	0/1	–	Negative
	30.51	2/4	0/2	Negative
	30.90	4/4	0/4	Negative
	32.23	1/4	0/1	Negative
	32.32	1/4	0/1	Negative
	32.53	3/4	0/3	Negative
	32.87	2/4	0/2	Negative
	33.82	1/4	0/1	Negative
	35.29	3/3	0/3	Negative
	30.62	0/4	–	Negative
Salchichón	33.47	1/1	1/1 (33.10)	**Positive**
	30.92	2/4	0/2	Negative
	31.63	3/4	2/3 (36.04, 34.95)	**Positive**
	33.33	1/4	1/1 (33.98)	**Positive**
TOTAL OF DRY-CURED SAUSAGES	**17 positive samples**	**3 infective samples**		

[a] Ct values of positive samples <38.

[b] Seropositive mice/Total of mice.
[c] Positive mice brain/Total of seropositive mice (Ct values of positive mice brains <38).

of samples analyzed (47/552). Of those samples, DNA of *Toxoplasma gondii* in mice brain was detected in 6 meat products, indicating its viability, which represents 1.1% (95%CI: 0.5%, 2.4%) of the total number of samples analyzed (6/552). Ct values obtained by qPCR in the brains of seropositive mice ranged from 33.10 to 36.04. The parasite was viable in 3 out of 311 dry-cured hams/shoulders (1.0%; 95%CI: 0.3%, 2.8%) and in 3 samples out of 241 dry-cured sausages (1.2%; 95%CI: 0.4%, 3.6%) (Table 2). All three samples of hams/shoulders in which *Toxoplasma* was viable came from white pigs, with curing times not exceeding 12 months. Regarding the three sausages, only one was an Iberian product. All samples, except one, in which the parasite was found to be viable were commercially available packaged slices. Although no corresponding information is available on the label, it is a customary technological practice to previously freeze the entire piece in order to facilitate the slicing process. If this technological procedure was indeed applied, it was obviously not sufficient to kill the parasite and consequently eliminate the risk. Actually, freezing does not always achieve total inactivation of the parasite (Gracia et al., 2022).

One of the first studies of commercial dry-cured meat products was carried out by Warnekulasuriya et al. (1998), who investigated *T. gondii* in 67 RTE cured meat samples collected by environmental health officers in London (United Kingdom), including dried and semi-dried sausages, fermented sausages, and cured (country-style) hams. Detection of *T. gondii* was determined by PCR and tissue culture was used in order to isolate viable parasites. They measured the pH and water activity of each meat sample, although the influence of those parameters was not evaluated. A sole sample of cured ham produced a positive PCR reaction as well as parasite-associated cytopathic effect on tissue culture, thereby indicating viability of the parasite: that result represents 1.50% of all tested samples, similar to our findings.

In a more recent study of 475 commercial *Serrano* ham samples (ham pieces and sliced ham), Gómez-Samblás et al. (2015) found a global prevalence of *T. gondii* of 8.8% (ranging from 0% to 32.35%, depending on the producer) applying magnetic capture real-time qPCR. The infectivity assays by mouse bioassay revealed that 4.8% of the positive samples were infective, slightly lower than our results. Costa et al. (2018) used qPCR to assess the presence of *T. gondii* DNA in 59 commercial cured salami samples from different producers in Brazil. They found 10 positive samples (16.9%). Sroka et al. (2019) detected the parasite in 14 out of 256 (5.5%) samples of dry-cured ham in Poland, less than our prevalence results in dry-cured ham/shoulders. Except for Gómez-Samblás et al. (2015), none of these authors assessed the viability of the parasites in the dry-cured meat products they analyzed.

Statistical analysis of our results showed that none of the label variables under study exerted a significant influence on the viability of the parasite. The non significance could be attributed to the very low number of viable samples (only 6). This agrees with the results obtained in a previous study (Herrero et al., 2017), in which several physicochemical parameters (moisture, water activity, fat, sodium chloride, nitrates and nitrites, and pH) did not have a significant influence on the viability of *T. gondii* in cured ham, except for fat content: lower fat content was associated with a significant ($p = 0.039$) loss of viability of *T. gondii* in dry-cured hams. This could be explained by the assumption that fat may protect tissue cysts from the effects of salt (Weiss & Kim, 2007).

On the other hand, Pott et al. (2013) studied the *in vitro* influence of physicochemical parameters (pH, sodium chloride, and sodium nitrite content) on the infectivity of *T. gondii*. They found that *T. gondii* tissue cysts have a high pH tolerance, but are very sensitive to salt. Moreover, they reported a stronger effect when sodium chloride was combined with 0.5% nitrite than when sodium chloride was applied alone. Furthermore, Fredericks et al. (2019) observed rapid inactivation of *T. gondii* bradyzoites in a low salt mass for dry-cured sausages within 4 h after the start of fermentation (pH 4.6–5.2 and $\geq$ 1.3% NaCl). Fredericks et al. (2020) demonstrated that a final salt concentration of 8.7–10.6% at 12 months, and a_w of 0.71–0.74, produced inactivation of *Toxoplasma*. These authors point out the great importance of salting and curing phase in the production of *T. gondii*-free cured pork products. Other authors have found that the inactivation of *T. gondii* during the curing process also depends on the synergistic interaction between salt concentration and curing time (Kijlstra & Jongert, 2008; Mie et al., 2008).

Regarding the effect of curing time on the inactivation of the parasite, scientific literature reports that longer curing times are related to lower viability rates and, consequently, contribute toward ensuring a lower risk for consumers. Bayarri et al. (2010) reported no viable parasites in 14 month-cured hams *vs* 7 month-cured hams. Gómez-Samblás et al. (2016) reported that at least 12 months of curing were required in dry-cured hams from white pigs to ensure a complete inactivation of the parasite. In Iberian dry-cured products, they did not detect infectivity in hams cured for 30 months, nor in shoulders cured for 20 months (Gómez-Samblás et al., 2021). Herrero et al. (2017) stated that viability was greater in hams cured for 9 months than in those cured for 12 months, although the latter duration resulted in a reduction, but not in a complete elimination of the risk. Conversely, Genchi et al. (2017) did not find viability by mice bioassay after 7 months of curing. They analyzed hams from experimentally infected pigs after curing times of 7, 12, and 14 months, according to standards for 'Parma Ham'.

In addition to the existence of varying elaboration processes for each meat product, it should be noted that a comparison of results obtained in all aforementioned studies is limited, due to the varying sampling and analytical methodologies used in each case. In the present study, we have used bioassay in mice for the detection of viable *T. gondii* in meat, a procedure regarded as the gold standard. In order to improve the method's sensitivity and to reduce the possibility of false negative results, we applied a concentration technique (acid pepsin digestion). False-negatives can also appear as a consequence of insufficient sample size or improper sample acquisition, since the number of *T. gondii* organisms in meat from naturally infected animals is very low, and organisms are inhomogeneously distributed (Opsteegh et al., 2020).

In conclusion, this study provides data on the prevalence of *T. gondii* in Spanish RTE dry-cured meat products (hams/shoulders, *chorizo*, *fuet/longaniza*, and *salchichón*) made commercially available to consumers. Results indicate a low prevalence of the infective forms of the parasite in cured meat products, although a certain risk cannot be completely excluded. This is particularly important if we consider the relevance of *Toxoplasma* as a biological risk in pork meat, the inability of current meat inspection procedures at slaughterhouse to detect the parasite, and the technological curing process's reported unequal effectiveness. Despite the inactivation parameters of *T. gondii* in dry-cured meat products, are well established by some authors, both in dry-cured fermented sausages and dry-cured hams, our study has shown that there are cured products available to the consumer in which the parasite is viable, with the consequent risk, especially for some groups of population. We believe that our results provide relevant data for the evaluation of the real risk for the consumer of this hazard agent in cured products. For all these reasons, monitoring of commercial meat products is required in order to continue providing scientific data for risk assessment of the parasite within the framework of the meat industry's HACCP-based self-control system. Validation and standardization of sensitive analytical methods are also required.

Finally, in order to provide consumers with the possibility of making a safe choice among RTE products, we find useful to include in food labels information on those parameters that are relevant for the survival of the parasite, such as curing times, or freezing treatment of meat used as an ingredient.

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CRediT authorship contribution statement

M.J. Gracia: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – review & editing. **P. Nieto:** Formal analysis, Investigation, Methodology. **R. Lázaro:** Data curation, Visualization, Writing – original draft, Writing – review & editing. **I. De Blas:** Data curation, Formal analysis, Writing – review & editing. **S. Bayarri:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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

The authors do not have permission to share data.

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