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María Jesús Gracia, Regina Lázaro, Consuelo Pérez-Arquillué, Rafael Pagán, Sergio Ramos, João Luis Garcia, Susana Bayarri

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TITLE:

High-Pressure Processing (HPP) of raw and dry-cured ham from experimentally infected pigs as a potential tool for the risk control of *Toxoplasma gondii*

Author names: María Jesús Gracia, Regina Lázaro[a], Consuelo Pérez-Arquillué[a], Rafael Pagán[a], Sergio Ramos[b], João Luis Garcia[c] and Susana Bayarri[a*]

Affiliation:

[a] Instituto Agroalimentario de Aragón - IA2 (Universidad de Zaragoza - CITA), Veterinary Faculty, 50013 Zaragoza (Spain)

[b] Centro Nacional de Tecnología & Seguridad Alimentaria CNTA, 31570 Navarra (Spain)

[c] Protozoology laboratory, Departamento de Medicina Veterinária Preventiva, Universidade Estadual de Londrina - UEL, Postal Box 6001, 86050-970 Londrina, PR, (Brazil)

***Corresponding author**: Dra. Susana Bayarri

Address: Dpto. Producción Animal y Ciencia de los Alimentos. Facultad de Veterinaria. Universidad de Zaragoza. C/ Miguel Servet 177, 50013, Zaragoza, Spain.

Phone number: +34-976-764135

Fax. number: +34-976-761612

E-mail: sbayarri@unizar.es

Abstract

Raw and dry-cured meats have been identified as a potential source of *Toxoplasma gondii* infection for humans. The present study evaluated the efficacy of an alternative non-thermal food-processing treatment, high hydrostatic pressure, on the viability of *T. gondii* bradyzoites in raw and dry-cured ham. Meat of pigs experimentally exposed to 4,000 oocysts of *T. gondii* VEG strain was vacuum-packaged and subjected to high pressure processing (HPP). Tap water (6ºC±1ºC) was used as the pressure-transmitting fluid, and its temperature during HPP increased 2.7ºC per 100 MPa. The effect was

evaluated by bioassay in mice followed by qPCR. In raw ham, 100-400 MPa/1 min did not inactivate *T. gondii*, whereas 600 MPa/20 min was effective. In dry-cured ham, 600 MPa for 3 or 10 min were not effective and a 20-min treatment was required to render the bradyzoites non-infectious for mice. Our results point toward the potential use of HPP as a tool for risk control of *T. gondii* and as a food safety guarantee.

Key words: *Toxoplasma gondii*; High-hydrostatic pressure processing; HPP; Pork meat; Dry-cured ham.

1. Introduction

Toxoplasma gondii is a food-borne parasite that causes human and animal toxoplasmosis. Human toxoplasmosis can be life-threatening in immunocompromised subjects. In addition, transplacental transmission during primary infection in pregnancy can result in blindness, congenital defects, mental retardation, or even fetal death (Robert-Gangneux, Aubert, & Villena, 2015). Furthermore, *T. gondii* infection has been implicated in different forms of behavioural alteration and neurological disorders in humans, suggesting that the infection is potentially more serious than previously supposed (Webster, Kaushik, Bristow, Glenn, & McConkey, 2013).

The main route of *T. gondii* transmission is via consumption of food and water contaminated with sporulated oocysts, or meat containing tissue cysts. Ingestion of undercooked or cured meat containing viable cysts has been suggested to be a major source of *T. gondii* infection (Buffolano et al., 1996; Cook et al., 2000; Jiang et al., 2018; Kapperud et al., 1996; Thaller, Tammaro, & Pentimalli, 2011).

Dry-cured ham is a ready-to-eat product (RTE) widely consumed in the Mediterranean area. It is a nonsmoked pork meat product cured with salt and nitrites, and stabilized through decreased a_w. The entire process takes at least 7 months, although in some cases the hams may be aged for more than 1 year. Curing has traditionally been regarded as an effective technology against *T. gondii* (Genchi et al., 2017; Franssen et al., 2019). Several studies have pointed out; however, that curing may not be enough to inactivate *T. gondii* bradyzoite cysts (Gómez-Samblás, Vilchez, Racero, Fuentes, & Osuna, 2015; Herrero et al., 2017; Warnekulasuriya, Johnson, & Holliman, 1998).

Additional treatment of meat to eliminate viable *T. gondii* tissue cysts would provide a means to protect consumers. Certain food-processing techniques, such as heat treatment, freezing, high hydrostatic pressure processing (HPP), and irradiation have parasite-inactivating potential (Franssen et al., 2019).

HPP is a technology that is receiving a great deal of attention worldwide. For the meat sector, HPP offers a valuable alternative to thermal pasteurization. Three main reasons make the HPP technology beneficial for meat products: the inactivation of pathogens such as *Listeria monocytogenes* or *Salmonella*, which allows the export of meat products to countries that require the absence of *L. monocytogenes*; an increase in shelf-

life by inactivating spoilage microorganisms; and the preservation of nutritional quality of raw and dry-cured meat without heat treatment (Bajovic, Bolumar, & Heinz, 2012; Hayman, Baxter, O'Riordan, & Stewart, 2004; Jofré, Aymerich, Grèbol, & Garriga, 2009; Rendueles et al., 2011).

The effectiveness of HPP treatment depends primarily on the applied pressure and on holding time. To pasteurize meat and meat products, pressure levels of 400-600 MPa and holding times of 3-10 min are applied at low temperatures, of 6-12ºC. In most cases, these treatments have led to an inactivation of more than four log units for the most common vegetative pathogenic and spoilage microorganisms, resulting in improved safety and increased shelf-life (Bajovic et al., 2012; Hugas et al., 2002; Hygreeva & Pandey, 2016; Rendueles et al., 2011). Although it is well known that the resistance of microorganisms is highly variable, depending on the type of organism and the food matrix involved (Rendueles et al., 2011), scant information is available on the subject of parasites in different food products, particularly in dry-cured ham.

Studies on the effectiveness of HPP in eliminating foodborne parasites have shown the sensitivity of *T. gondii* (Lindsay, Collins, Jordan, Flick, & Dubey, 2005; Lindsay, Collins, Holliman, Flick, & Dubey, 2006; Lindsay et al., 2008), *Cryptosporidium parvum* (Collins et al., 2005), *Anisakis* larvae (Brutti et al., 2010; Molina-García & Sanz, 2002), *Trichinella spiralis* (Noeckler, Heinz, Lemkau, & Knorr, 2001), *Ascaris* (Rosypal, Bowman, Holliman, Flick, & Lindsay, 2007), and *Trichuris vulpis* (Rosypal, Zajac, Flick, Bowmand, & Lindsay, 2011) in low pressure ranges (100-400 MPa).

HPP could therefore be an appropriate tool for reducing the risk of *T. gondii.* Although previous studies have indeed evaluated the effect of HPP on oocysts of *T. gondii* (Lindsay et al., 2005, 2008), little has been published on the effects of HPP on tissues infected with *T. gondii*. Regarding raw meat, only the study conducted by Lindsay et al. (2006) has shown that *T. gondii* tissue cysts in ground pork have been successfully inactivated by applying 300-400 MPa for 30 s, whereas 100 and 200 MPa have been ineffective. The tissues, however, did not come from infected animals (naturally or experimentally), but had been intentionally spiked with parasites.

The properties of the food matrix have been shown to have a great influence on the lethality of HPP. Dry-cured ham, a salted and dried product with low a_w, has shown lower inactivation levels of some pathogens (*Salmonella*, *L. monocytogenes*, and *S. aureus*) and spoilage (lactic acid bacteria) microorganisms after HPP than other meat products (Jofré et al., 2009; Rendueles et al., 2011).

To the best of our knowledge, no previous studies evaluating the effect of high pressure on dry-cured meat parasitized with *T. gondii* have been carried out to date. Therefore, the objective of the present study was to evaluate the potential use of the actual HPP treatments commonly employed by the meat industry for risk control of *T. gondii* in dry-cured ham. For this purpose, we evaluated the effects of HPP on the viability of *T. gondii* bradyzoites infecting raw and dry-cured ham made from experimentally infected pigs.

2. Material and methods

2.1. Animal infection, raw and dry-cured ham sampling, and sample preparation for analysis

Two *T. gondii* seronegative white pigs were used (~50 kg, 5 months of age). One pig was infected orally with 4,000 sporulated *T. gondii* oocysts (VEG strain). The other pig was left uninfected and was used as the negative control. Animal infection and slaughter was approved by the Ethics Committee of the Complutense University of Madrid (Ref. PROEX 415/15). The pigs were housed and maintained under Biosafety Class III conditions (in the VISAVET facilities of the Veterinary Faculty of Complutense University of Madrid) until the study was completed. Sixty days post infection, the animals were slaughtered and both haunches were collected. One haunch was analysed on day zero, and the other was cured for 12 months. The technological treatment of the ham was carried out in a cured ham processing facility as described by Herrero et al. (2017). Water activity was determined in final dry-cured hams by the Association of Official Analytical Chemists Official Method 978.18 (AOAC, 1998). Boneless raw and dry-cured ham were cut into small pieces, minced, homogenized and refrigerated.

2.2. High pressure processing

Fifty grams of prepared samples were placed in sealable plastic bags and vacuum-packaged. The sealed bags were then placed in additional sealable bags and sealed again. These additional bags were used to prevent any potential contamination of the HPP unit. Samples were stored under refrigeration until HPP treatment and analysed thereafter.

HPP process was performed in an industrial Hiperbaric 135 high pressure processing unit (Hiperbaric Burgos, Spain), equipped with a 135 L vessel, using tap water (6ºC±1ºC) as the pressure-transmitting fluid. The water temperature during HPP increased 2.7ºC per 100 MPa.

A series of different HPP treatments were carried out. HPP was applied to raw meat at 100, 200, 300, and 400 MPa with a holding time of 1 min at each pressure tested. In addition, a HPP treatment of 600 MPa was applied during a holding time of 20 min. High hydrostatic pressure was applied to dry-cured ham at 600 MPa with holding time of 3, 10, and 20 min. Each HPP treatment was replicated three times. Pressure buildup was 240 MPa/min, and decompression was instantaneous.

A negative control and a positive control were included for each assay (raw and dry-cured ham). The negative control did not contain *T. gondii* cysts, while the positive control did, but was not subjected to any HPP treatment.

2.3. Mouse bioassay of tissues for T. gondii

To determine viability, we performed bioassay on mice, followed by real time PCR (qPCR). After the inoculation to mice of the sample to be analysed, seroconversion

occurred if the parasite was contained in it. If the parasite was viable in the pork sample, the tissue cysts could form in the brain of exposed mice (Figure 1) and tissue cyst formation could be confirmed by PCR. For each HPP-treated or control sample, a concentration bioassay technique with an acid pepsin digestion procedure was applied to raw and dry-cured ham, as described elsewhere (Bayarri et al., 2010; Dubey, 1998). 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). All experiments included negative control mice. The mice came with a health certificate attesting that they were free from pathogens. They were kept at the Centro de Investigación Biomédica de Aragón (CIBA) in Zaragoza (Spain). The 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).

2.3.1. IFA of mouse sera

Blood samples were drawn from mice that survived 60 days after inoculation. Sera samples of mice were analysed by IFA to detect antibodies against *T. gondii* with polyclonal rabbit antimouse immunoglobulins (DakoCytomation). Serum from each mouse was diluted 1:10, 1:20, 1:40, 1:80, 1:160, and 1:320. A positive and a negative control serum from previous studies in our laboratory were included in each test. Final preparations were examined with an Eclipse 80i fluorescence microscope (Eclipse 80i, Nikon instruments INC, Netherlands). Sera samples with a titer of 1:10 were considered positive.

2.3.2. DNA extraction and identification of T. gondii

Analysis of *T. gondii* DNA from brains of serologically positive mice was performed by qPCR to determine viability of the parasite. Fifteen mg piece of each brain were homogenated through hand pistel plastic rotating plungers in an eppedorf. The DNA extraction was performed using UltraClean® Tissue & Cells DNA Isolation Kit Sample Catalog No. 12334-S (Mobio Laboratories, Inc.) according to the manufacturer's instructions. Two sets of primers for DNA amplification targeting specific sequence of 529 repeat element were used (ToxoRoc F TAGACGAGACGACGCTTTCC, ToxoRoc R TCGCCCTCTTCTCCACTCT, and ToxoRepeat 500 F CGCTGCAGGGAGGAAGACGAAAGTTG, ToxoRepeat 500 R CGCTGCAGACACAGTGCATCTGGATT) and another set of primers for the surface antigen marker genes (SAG) (ToxoSG1 F TCATCGGTCGTCAATAA, ToxoSG1 R CTTTGACTCCATCTTTCC). A CFX Connect real time PCR instrument (Bio-Rad Laboratories) was used for the amplification and detection of *T. gondii* using Gotaq Sybergreen Master Mix (catalogue # A6002) from Promega. The reaction volume was 20 ml, and samples were run in triplicates. The protocol consisted of 7 min at 94 ºC for enzyme activation (hot start), and 40 cycles of denaturation at 94 ºC for 5 s, annealing at

55 ºC for 30 s, and extension at 72 ºC for 10 s. The program ended with a dissociation curve from 60 to 94 ºC with a 0.5 ºC increase interval. Each PCR run included a negative control, a positive control, and a separate reaction for Actin DNA copies as internal control (IC). A sample was considered positive if at least two of the triplicates were positive with both markers. The threshold cycle (Ct*)* value used was indicated by the marker 529 repeat element (Ct must be lower than 38), and the SAG marker served to confirm the result. Calibration curves were prepared using 15 mg of homogenate negative tissues spiked with a known number of tachyzoites (10^4, 10^3, 10^2, 10^1, and 10^0). The samples were subsequently homogenized and processed with the commercial DNA extraction kit following the manufacturer's instructions in the same way as the rest of the samples.

3. Results and Discussion

The results of the effect of high-pressure processing on *T. gondii* tissue cysts in raw ham are presented in Table 1. High-pressure processing of raw meat at pressures of 100, 200, 300, and 400 MPa with holding time of 1 min was not effective in reducing infectivity of *T. gondii*. Treatment with HPP at 600 MPa for 20 min completely eliminated infectivity for mice. None of the mice inoculated with non-infected raw meat became infected with *T. gondii.* Infection was observed in mice inoculated with non-pressure-treated *T. gondii* tissue cyst containing ground pork.

Our results differ from those described by the only study that has previously evaluated the application of HPP treatments against *T. gondii* inoculated in raw meat (Lindsay et al., 2006). According to those authors, the HPP treatment has achieved the inactivation of the parasite with pressures at 300-400 MPa and a holding time of 30 s. These results, differing from ours, could be due to the fact that the authors have worked with ground pork intentionally spiked with mouse brains containing viable tissue cysts; later samples have been digested in acid pepsin solution and bioassayed in mice. Since the parasite is contained in the mouse brain tissue and not in pig muscle, it is possible that the digestion process may have affected the viability of bradyzoites to a greater extent (Dubey et al., 1995; Dubey, 2010; Mendoça, Domingues, da Silva, Pezerico, & Langoni, 2004). Our study, on the contrary, used experimentally infected animals, which implies that *T. gondii* tissue cysts were located in the muscle tissue, which is more resistant to the digestive process. As the first investigation to evaluate HPP in meat from animals experimentally infected with *T. gondii*, this study holds special relevance, since it more faithfully reproduced the conditions in which the parasite is found within the muscular tissues.

Another possible reason for our discrepancy with the results of the research carried out by Lindsay et al. (2006) is the technique we used to confirm viability. Bioassay is regarded as the gold standard for the evaluation of treatment efficacy of parasites in food (Franssen et al., 2019). Both Lindsay et al. (2006) and our own study have used bioassay; however, we confirmed viability by analysing the mouse brain by q-PCR, unlike Lindsay et al. (2006), who have used histology. PCR has more specificity and

sensitivity in detecting the presence of *T. gondii* than histological detection (Esteban-Redondo et al., 1999; Opsteegh, Schares, & van der Giessen, 2016). Research on foodborne parasites should be improved with the goal of standardizing experimental approaches for the evaluation of inactivation methods, but also in order to standardize methods designed to monitor inactivation (Franssen et al., 2019).

In the course of the HPP process, pressure is transmitted uniformly and instantly (Hugas, Garriga, & Monfort, 2002) with little variation in temperature, independent of food shape or size (Rendueles et al., 2011). This aspect is of particular interest when investigating pathogens such as *T. gondii*, for which the distribution of cysts in tissues is thoroughly random. It has been estimated that the number of *T. gondii* cysts per gram of tissue from food animals such as pigs may be less than 1 cyst/50 g of tissue, and one cyst may contain a few to 1000 individuals (Esteban-Redondo et al., 1999; Opsteegh et al., 2016).

High hydrostatic pressure usually exerts a stronger destructive effect on organisms with a higher degree of organization and structural complexity. Prokaryotes are usually more resistant than eukaryotes (Yuste, Capellas, Fung, & Mor-Mur, 2001), and it is assumed that the destruction of parasites is achieved with relatively low pressures (Rendueles et al., 2011). However, the pressures and times required to inactivate *T. gondii* are higher than those used in previous studies against tissue cyst (Lindsay et al., 2006) or oocysts (Lindsay et al., 2008). They are also superior to those used against other parasites (Brutti et al., 2010; Collins et al., 2005; Molina-García & Sanz, 2002; Noeckler et al., 2001; Rosypal et al., 2007, 2011).

The application of HPP to meat modifies quality parameters such as colour, texture, and water holding capacity (Bajovic et al., 2012). Meat colour is one of the most important quality characteristics for consumers in a purchase situation. HPP affects quality parameters of raw meat, particularly depending on the pressure level applied, and thus typical characteristics associated with raw meat such as texture and, especially, colour can be modified to a remarkable degree. The meat becomes more gel-structured and paler, losing the typical appearance of raw meat (Bajovic et al., 2012). Thus hampers the commercialization of HPP-treated raw meats due to the loss of the typical raw meat colour from a consumer perspective (Cheftel & Culioli, 1997). These changes are indeed relevant if the products are further processed or even directly consumed; they would, however, be safer and more nutritious than cooked meat, since HPP has no negative impact on nutritional value (Bajovic et al., 2012). Regarding the physicochemical effect of HPP technology on food, it is softer and preserves flavour better than a thermal treatment (Schindler, Krings, Berger, & Orlien, 2010). Such changes in colour and texture may even be desirable in some cases, when the purpose is to elaborate new products and new forms of presentation.

The chemical composition of food has been identified as an important factor that can influence the greater or lesser resistance of microorganisms to HPP. The efficacy of

HPP decreases with reduced a_w (Jofré et al., 2009; Rendueles et al., 2011), as is the case of the dry-cured ham samples evaluated in this study (a_w ~0.85).

For our study of the effect of HPP on *T. gondii* tissue cysts contaminating dry-cured ham, we assayed three different HPP treatments. We first applied two treatment conditions (600 MPa/3 min and 600 MPa/10 min) commonly used by the food industry to control *L. monocytogenes* and fulfil the requirements of USDA and Health Canada (Bover-Cid, Belletti, Garriga, & Aymerich, 2011; Hayman et al., 2004; Hugas et al., 2002). These holding times are directly related with the water activity and fat content of the dry-cured products and, depending on these two characteristics, HPP is modulated between 3 and 10 min of treatment (habitually 5 min) (Hereu et al., 2012). We then applied a longer treatment, of 600 MPa/20 min. As described in Table 2, while HPP at 600 MPa for 3 and 10 min did not eliminate *T. gondii* infectivity for mice, 600 MPa for 20 min guaranteed its inactivation. None of the mice inoculated with non-infected dry-cured meat became infected with *T. gondii*. Conversely, infection was observed in mice inoculated with non-pressure-treated *T. gondii* tissue cyst containing ground pork.

Such extended times do not tend to be employed at the industry (Bajovic et al., 2012; Hygreeva & Pandey, 2016; Rendueles et al., 2011); instead, times of 3-10 min are more common (Bover-Cid et al., 2011; Hayman et al., 2004; Jofré et al., 2009). Six hundred MPa for 10 min of holding time have been necessary to reach 3 log-inactivation of pressure-resistant *L. monocytogenes* in Spanish dry-cured ham (a_w 0.880; pH 5.84) (Bover-Cid et al., 2011). This inactivation level is relevant for fulfilling the requirements of USDA and Health Canada regarding the control of *L. monocytogenes* in RTE food products. To the best of our knowledge, ours is the first study to have evaluated the effect of HPP on the inactivation of *T. gondii* in dry-cured ham from experimentally infected animals.

It is generally accepted that cured meat colour is particularly resistant to HPP. The majority of studies have reported no change in redness, thereby supporting the protective role of nitrification on meat colour (Bak, Bolumar, Karlsson, Lindahl, & Orlien, 2019). The colour of meat is strongly related to its water content (Ferrini, Comaposada, Arnau, & Gou, 2012), and HPP applied for up to 10 min have had a negligible effect on dry-cured ham. However, with more extended treatment times, visible changes could appear in the products (Bajovic et al., 2012; Bak et al., 2019). These changes might vary according to the meat's initial textural characteristics and depending on its protein system, its temperature, the pressure applied, and the duration thereof. Juiciness, springiness, and chewiness are increased by HPP depending on treatment time (Coll-Brasas et al., 2019). Longer treatments increase production costs, since they limit the number of treatment cycles per hour and reduce the kilo production rate per hour. In order not to exceed those times, one of the solutions applied both in the meat sector and in other sectors (juices and beverages, prepared dishes, etc.), consists in applying multi-pulsed pressure cycles (compression and decompression rates). Multi-pulsed HPP could be used to inactivate spoiling and pathogenic agents, thereby helping to improve food safety and quality. An optimized balance between ideal pressure,

temperature, pulse number, pressure holding time, and compression and decompression rates can increase the effectiveness of such treatments. Depending on the product, such an application of pressure pulses could be more effective than a longer continuous treatment, since the same level of inactivation could be attained while achieving certain gains in terms of sensory quality (Buzrul, 2014). More studies are needed to evaluate the costs and the technical characteristics of the equipment required to apply multi-pulsed HPP treatments. It would likewise be interesting to evaluate shorter holding times at 600 MPa to ascertain their effectiveness. Further research is needed to evaluate the applicability of HPP to inactivate *T. gondii* in food of animal origin.

4. Conclusions

In conclusion, both raw and dry-cured ham treated for 20 min with HPP at 600 MPa were rendered free of viable *T. gondii* tissue cysts, thereby confirming high hydrostatic pressure technology as an alternative capable of guaranteeing the commercialization of safe meat products. Further research is needed to evaluate other HPP process conditions in the inactivation of *T. gondii* in food of animal origin.

Declarations of interest: none

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5th December 2019

Author statement

Dear editors,

Please, below you can find the individual contribution of authors to the paper IFSET_2019_1177, using CRediT roles:

Susana Bayarri, as coordinator of the research project leading to this publication,has proposed the study, has dealt with the acquisition of the financial support, as well as the planning, execution and supervision of the research activity. She has coordinated the multidisciplinary team integrated by the authors of this work. (CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Visualization; Writing - review & editing).

María Jesús Gracia, parasitology expert, has proposed the study and has participated on the planning of the trials. She has also contributed to experimental tasks related to pig infection and sample analysis (mouse bioassay). She has been responsible for writing the initial draft. (CRediT roles: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing - original draft; Writing - review & editing).

João Luis Garcia, parasitology expert, has provided the *Toxoplasma* VEG strain for the study and has participated in the experimental pig infection design. (CRediT roles: Conceptualization; Formal analysis; Investigation; Methodology).

Rafael Pagán and **Sergio Ramos** have provided expertise in food technology and instrumentation for HPP treatment, and have performed HPP experiments. (CRediT roles: Conceptualization; Formal analysis; Investigation; Methodology; Visualization; Writing - review &editing).

Regina Lázaro and **Consuelo Pérez-Arquillué**, food safety experts, have participated in sample preparation for HPP treatments and have contributed to data analysis related with risk assessment. (CRediT roles: Conceptualization; Investigation; Methodology; Visualization; Writing - review &editing).

Sincerely yours,

Susana Bayarri (Corresponding author)

Figure 1. *Toxoplasma gondii* cyst in mouse brain

Table 1. Effect of different HPP treatment conditions on *Toxoplasma gondii* tissue cysts in raw ham

Treatment	**Exposure time (min)**	**Seropositive mice [a]**	**Viability of *T. gondii* [b]**	**Infectivity of the sample**
Negative control	0	0/8	-	-
Positive control	0	4/8	4/4 35.61 33.83 34.23 35.02	+
100MPa	1	2/8 8/8 5/8	1/2 35.29 2/8 31.01 31.43 1/5 35.75	+
200MPa	1	2/8 2/8 3/8	0/2 0/2 2/3 33.20 31.45	+
300MPa	1	4/8 4/8 5/8	2/4 32.46 32.42 0/4 2/5 34.78 31.51	+
400MPa	1	2/8 3/8 2/8	2/2 33.88 32.35 0/3 0/2	+
600MPa	20	2/8 3/8 3/8	0/2 0/3 0/3	-

[a]Seropositive mice by IFA/Total of mice.

[b]Positive mice brain by PCR/Total of seropositive mice by IFA; Ct values of positive mice brains (< 38).

Table 2. Effect of different HPP treatment conditions on *Toxoplasma gondii* tissue cysts in dry-cured ham

Treatment	**Exposure time (min)**	**Seropositive mice** [a]	**Viability of *T. gondii*** [b]	**Infectivity of the sample**
Negative control	0	0/8	-	-
Positive control	0	5/8	3/5 28.77 29.77 30.58	+
600MPa	3	5/8 3/8 3/8	0/5 0/3 1/3 34.84	+
600MPa	10	4/8 2/8 1/8	1/4 34.67 0/2 0/1	+
600MPa	20	1/8 1/8 4/8	0/1 0/1 0/4	-

[a]Seropositive mice by IFA/Total of mice.

[b]Positive mice brain by PCR/Total of seropositive mice by IFA; Ct values of positive mice brains (< 38).

Highlights

- First HPP study on *T. gondii* in raw and cured ham from experimentally infected pigs
- Viability of *T. gondii* assessed by bioassay in mice followed by PCR
- 600 MPa/10 min does not inactivate *T. gondii* in dry-cured ham while 600 MPa/20 min does
- HPP of raw and dry-cured ham is a potential tool for the risk control of *T. gondii*

Industrial relevance text

Under real production conditions, the usual HPP treatments applied by the food industry to control *L. monocytogenes* and other pathogenic bacteria are 600 MPa of pressure and holding times of 3-10 min. Our study demonstrated, however, that longer treatment times are required to inactivate the parasite and, thus, to guarantee the safety of raw and dry-cured meats in order to reduce the public health risk of toxoplasmosis. Further research is needed to evaluate other HPP conditions, including pulsed cycles, for the inactivation of *T. gondii* in foods of animal origin.

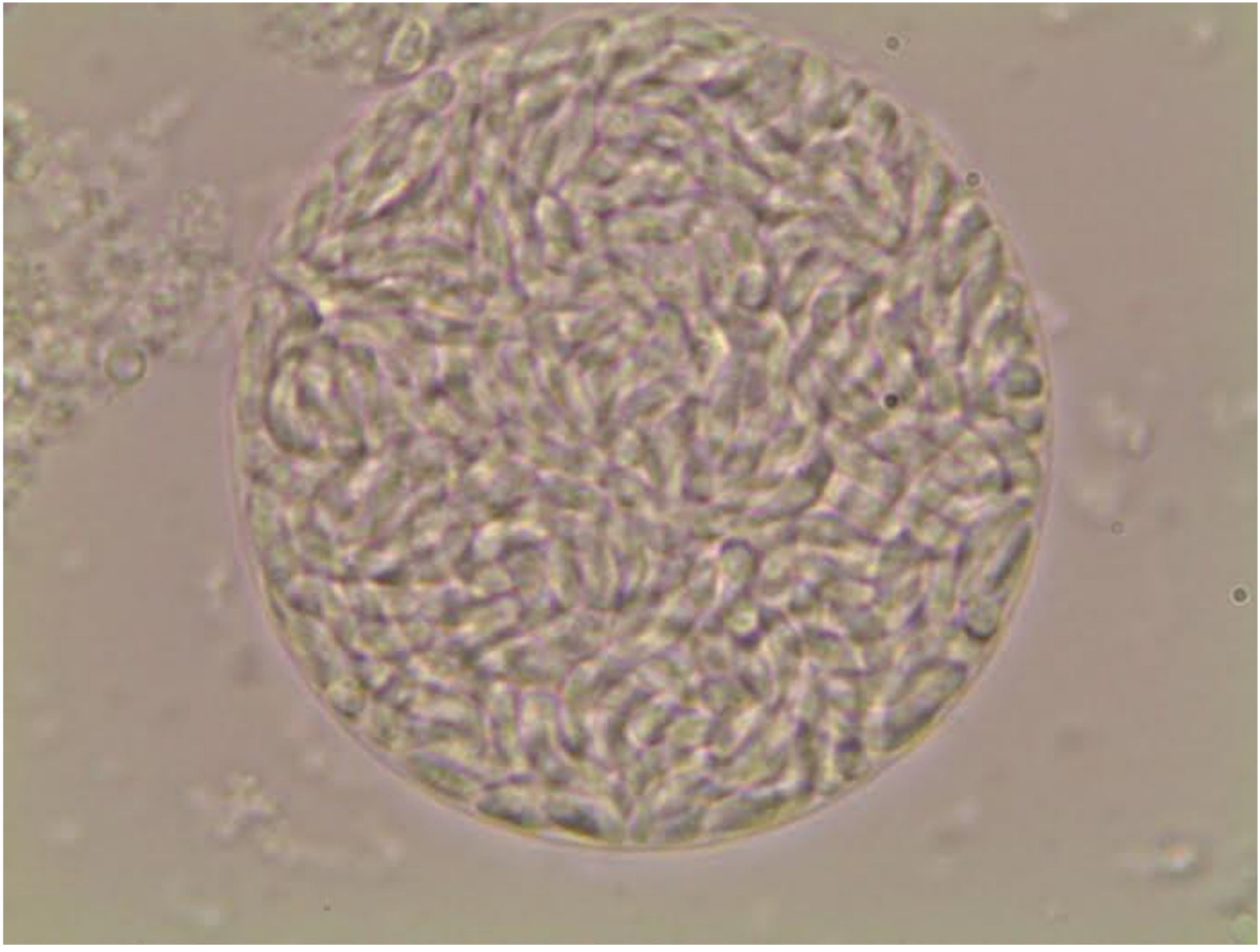

Figure 1