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Survival of experimentally induced *Toxoplasma gondii* tissue cysts in vacuum packed goat meat and dry fermented goat meat sausages

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ABSTRACT

Ingestion of raw or undercooked meat is a potential source of human toxoplasmosis. The aim of this study was to determine the viability of *Toxoplasma gondii* cysts in vacuum packed (VP) goat meat and in dry fermented sausages (DFS), and evaluate certain physical and chemical parameters, like water activity (a_w), pH value, content of salt, dry matter and fat. A portion of muscle tissue from experimentally infected animals was used for production of VP meat with or without addition of 2.5% curing salt, and stored at 4 °C or at −20 °C. Results of bioassay showed that, samples of vacuum packed *Toxoplasma* positive meat without salt addition were alive after six weeks at 4 °C. Incubation at −20 °C supported the viability after 3 h, but not after 4 h. After 7 days in 2.5% of curing salt, samples of *T. gondii* VP goat meat were still viable, but not after 14 days at 4 °C. All the DFS samples were not positive for infective cysts which mean that, they do not pose a risk of *T. gondii* transmission. These data suggest that vacuum packaging increases the survival of *T. gondii* cysts.

1. Introduction

Toxoplasma gondii is a heteroxenous coccidian parasite of humans and warm-blooded animals with a worldwide distribution that can cause serious illness, being dangerously virulent in immunocompromised individuals and congenitally infected children. The sexual part of the life cycle occurs in feline carnivores, which excrete the oocysts in their faeces. After a 2–5 day long sporulation process, the oocysts become infectious and can be transmitted to other hosts through inadvertent ingestion. Asexual multiplication in the intermediate host leads to the formation of tissue cysts in muscle tissue and organs, which are infectious for hosts that consume them, including humans (Dubey, 2004). Whether tissue cysts are the actual source of infection is usually not known, but there are several reports of *T. gondii* induced outbreaks that have been associated with the consumption of raw meat in Canada, Korea, French Guiana and New Zealand (McDonald et al., 1990; Choi et al., 1997; Carme et al., 2002; Lake et al., 2002).

After ingestion of tissue cysts by the intermediate host bradyzoites transform to tachyzoites, multiply locally, and disseminate in the body. In pregnant females there is even possible transfer to the foetus through the placenta. In many studies, ingestion of inadequately cooked meat was linked to *T. gondii* infection in pregnancy (Kapperud et al., 1996; Bobić et al., 1998; Cook et al., 2000). However, while the consumption of raw or undercooked meat was consistently identified as a risk factor for toxoplasmosis in many studies, its relative importance and the meat type varied among different countries (Cook et al., 2000).

Many serological studies have been performed in livestock, but this line of research does not provide a true assessment of the toxoplasmosis risk for humans as the packing and storage of meat also affects the viability of *T. gondii* tissue cysts. In several studies, the susceptibility of cysts to various physical traumas such as heat treatment, freezing, gamma irradiation or high-pressure, was tested (Dubey et al., 1990; Kuticic and Wikerhauser, 1996; Lindsay et al., 2006). Heat treatment is the most secure way to inactivate tissue cysts while bradyzoites are also destroyed by salting, curing, pickling and the use of enhancing solutions, like sodium chloride, potassium lactate, sodium lactate etc., which can be injected in to ensure longer stability or better taste of final meat product, but some of these

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treatments have not been standardized (Kotula et al., 1991; Lundén and Uggla, 1992; Hill et al., 2006). Vacuum packaging of meat is commonly used to preserve meat cuts and until now there have been no studies accessing the viability of *T. gondii* after the vacuuming process, which facilitates the longer shelf-life of the meat. In addition, dry fermented meat products have also been implicated in the epidemiology of toxoplasmosis, because there is no thermal treatment step during this manufacturing process. The production technology of dry fermented meat products is unique because of the number of biochemical processes that takes place during the ripening phase, which contributes to the preservation of the final product.

Among the various kinds of commonly consumed meat, goat products pose a risk of infection due to their high susceptibility to *T. gondii*, as exemplified by its high prevalence which ranges up to 77% of the meat on the market in some countries (Dubey and Beattie, 1988; Tenter et al., 2000; EFSA, 2007; Dubey et al., 2011; Hill and Dubey, 2013). The assessment of risk depends not only on the presence of antibodies against *T. gondii* but also on the parasite quantity in meat. Although some studies have reported the isolation of *T. gondii* from caprine tissues and their consequent genotyping (Dubey et al., 2011), the level of infection was not quantified. Recently, Juránková et al. (2013) described the distribution of *T. gondii* parasites in meat cuts and organs of experimentally infected, post-weaned goat kids. All tested muscles from shoulder, loin and leg, were found to be positive for the parasite, and the highest parasite load was in the dorsal muscle tissue of goats euthanized 90 dpi. This study directly builds on this previous work. Its main aim was to access the viability of *T. gondii* cysts in VP[1] meat from experimentally infected goats stored at different conditions and after the manufacturing of DFS[2]. Because past studies have lacked detailed meat product characteristics, another goal was to test the physical and chemical parameters like water activity, the activity of the hydrogen ion (pH), content of salt, dry matter and fat of VP goat meat and DFS to define their influence on *T. gondii* bradyzoites survival.

2. Materials and methods

2.1. Experimental design

After experimental infection of naive goat kids, we obtained from them meat containing *T. gondii* bradyzoites. Infected goat meat was either processed by vacuum packaging, with or without curing salt addition, or made into fermented sausages. All the samples after these different treatment and storage conditions were used in bioassays of four outbred mice to determine *T. gondii* cyst viability. The testing schemes of vacuum packed meat and dry fermented sausages are shown in Figs. 1 and 2, respectively.

2.2. Experimental infection

Meat from sixteen shorthaired male post-weaned 64–68 day old kids, which were experimentally infected with a suspension of 20,000 oocysts of *T. gondii* of genotype II, Apico (Juránková et al., 2013) was used in this study. Animals were housed in the animal care facility at the Ruminants and Swine Clinic of the University of Veterinary and Pharmaceutical Sciences Brno and were handled with the agreement of the Ethical Commission. The euthanasia was performed after intravenous injection of thiopental and cutting jugular veins after passing out. The meat cuts were collected and confirmed as *T. gondii* positive. The experimental infection of goats is described in detail in Juránková et al. (2013).

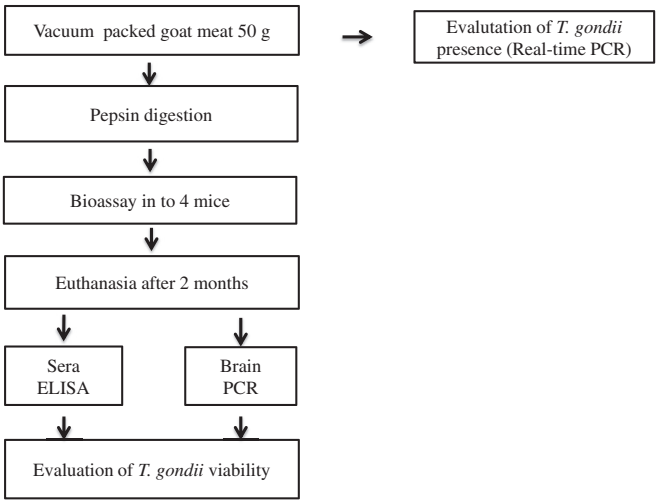

Fig. 1. Testing scheme for vacuum packed goat meat.

2.3. Production of vacuum packed goat meat

Infected meat from shoulders, loins and legs were collected and cut into small pieces of about 1 × 1 cm in size. Fifty grams of these cubes were individually packaged into bags. These bags consisted of a 60 μm coat of polyamide (PA), 20 μm coat of polyethylene (PE) with an ethylene-vinyl acetate (EVA) oxygen barrier layer (AMILEN PA/PE, Verpackungen GmbH, Germany). The manufacturer declares this barrier having a gas transmission rate of 50, 10 and 150 $cm^3\ m^{-2}\ d^{-1}$ permeability for O_2, N_2 and CO_2, respectively, at 23 °C, 0% relative humidity. According to manufacturer, the water vapour transmission rate was 3.0 $g\ m^{-2}\ d^{-1}$ at 23 °C, 85% relative humidity. Weight per area was 80 $g\ m^{-2}$, tensile strength at break 45% longitudinally and 35% transversally. After being filled with meat samples the 99.9% of the air was evacuated from the package, sealed using of a Vac-Star vacuuming packaging machine (S 223 GX, Frimark CZ Ltd., Czech Republic). Vacuumed packages of goat meat with the addition of 2.5% curing salt (sodium nitrite 6% and sodium chloride 94%) or unsalted were stored at 2 ± 2 °C and stored for a periods of 2 h as well as 7, 14, 21, 36 and 42 days. Other packages of unsalted meat were stored at −20 °C for 30 min as well as 1, 2, 3, 4, 5 and 6 h. The temperature of stored meat (2 ± 2 °C) was

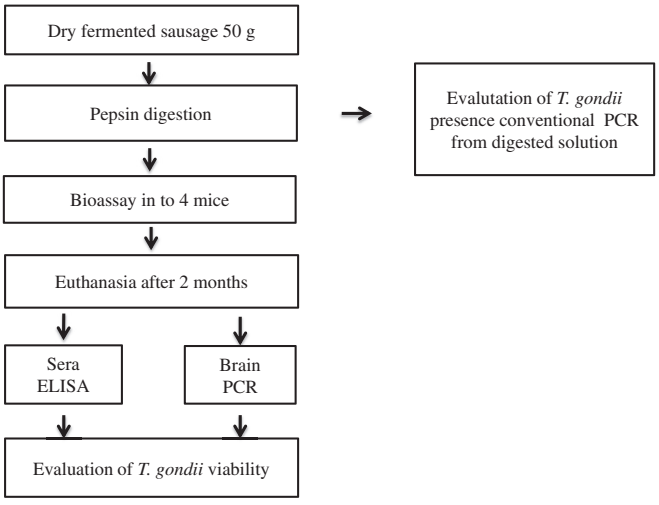

Fig. 2. Testing scheme for dry fermented sausages.