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Research Article

INVESTIGATION OF *TOXOPLASMA GONDII* AND *SARCOCYSTIS* SPP. TISSUE CYSTS IN MEAT PRODUCTS AVAILABLE TO THE MARKET BY PERCOLL GRADIENT METHOD

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ABSTRACT. Purpose of this study is microscopic examination of *Toxoplasma gondii* and *Sarcocystis* spp. in lamb meat, beef, meatball and sausages launched for retail sale in Ankara and Kırıkkale using Percoll Gradient method. By visiting butchers in Ankara and Kırıkkale 50 pieces of each meat products (200 total) were purchased. After 5 grams of the samples were homogenized, they were subjected to the Percoll gradient method. Ten coverslip areas from each sample were examined under the light microscope for the presence of *T. gondii* and *Sarcocystis* spp. tissue cysts. In 78.5% of the 200 samples that examined there was at least one parasite species tissue cyst. In 2.5 % of total samples had *T. gondii* and 56.5% had *Sarcocystis* spp. and 19.5% had both *T. gondii*+*Sarcocystis* spp. tissue cysts. 95% of lamb meats, 62% of beef, 90% of meatballs and 68% of sausages were found to be infected with at least one species. In conclusion, *T. gondii* and *Sarcocystis* spp. tissue cysts were found on meat products that launched in Kırıkkale and Ankara. Because of the zoonotic feature of *T.gondii* it is highly important to consume meat products after cooked well for public health. And for prevent meat products from getting *Sarcocystis* spp. tissue cysts, it is important to take cautions for our animals to not get infected.

Keywords: *Meat, percoll gradient, sarcocystis, tissue cyst, Toxoplasma gondii.*

INTRODUCTION

Toxoplasma gondii is the only species in the Toxoplasmatidae family [1]. Felines are both intermediate and final hosts of this parasite; however, many mammals including humans and poultry are intermediate host of this parasite. Humans are infected by consuming raw or undercooked meat and ingesting sporulated oocysts of the parasite through food and water. Congenital transmission is the other important route of transmission in humans. *Toxoplasma gondii* causes abortions in farm animals, especially in sheep and goats [2]. In humans, abortion in seronegative pregnant women causes serious health problems up to death in immunocompromised people [1, 3].

Sarcocystis species are apicomplexan protozoan parasites with intracellular localisation [4]. These species need two hosts to complete their life cycle. Humans and carnivores are the final hosts of these parasites, and herbivores are the intermediate hosts [5]. These species are commonly found in butchery animals, poultry, some reptiles and

humans. Macro and microcysts of these parasites are found in the skeletal muscles, heart, oesophagus, diaphragm, tongue and masseter muscles of the host [6]. Sarcocystosis causes nausea, abdominal pain and diarrhoea in humans [7]. In farm animals, it causes weight loss, abortion, anaemia and death in severe infections [8].

Contaminated raw or undercooked farm animal meat, unpasteurised goat milk and *T. gondii* contaminated vegetables, fruits and water are sources of *T. gondii* for humans [9]. Transmission of *Sarcocystis* species to humans occurs by consuming raw or undercooked pork and beef infected with zoonotic species [10].

The aim of the present study was to investigate the tissue cysts of *T. gondii* and *Sarcocystis* spp. in lamb, beef, butcher meatballs and sausage samples offered for sale in Ankara and Kırıkkale by using Percoll gradient method. Tissue cysts of *Sarcocystis* spp. have been tried to be detected in the tissues of various animal species by using various digestive techniques. However, studies on the detection of tissue cysts of *T. gondii* and *Sarcocystis* species by Percoll gradient method are very limited.

MATERIALS AND METHODS

A total of 200 samples (50 from each of lamb, beef, sausage and butcher meatballs) were taken from butcher shops and markets in Ankara and Kırıkkale. The samples were transported to Routine and Epidemiology Laboratory of Parasitology Department within Veterinary Medicine Faculty of Kırıkkale University in accordance with the cold chain rules. Five grams of each sample was weighed in the laboratory. The weighed samples were placed in sterile plastic containers and 20 ml phosphate buffer saline (PBS) was added. The samples were crushed with a homogeniser (Omni Tip, USA) until achieving complete homogenization. The resulting solution was filtered through a strainer into centrifuge tubes and centrifuged at 500 g for 20 minutes. At the end of centrifugation, the supernatant was discarded with a Pasteur pipette. Freshly prepared 90% and 30% Percoll solution were added to a centrifuge tube to form a layer respectively. On this solution, 4 ml of homogenised homogenate from each sample was added slowly so as not to mix with the Percoll solution. These tubes were centrifuged at 4000 g for 20 minutes to collect the tissue cysts. After centrifugation, the area between the percoll dilution layers was gently removed with a sterile Pasteur pipette and transferred to a 1.5 ml microtube [11]. Ten slide fields from each sample were examined under a light microscope (Leica DM750) for *T. gondii* and *Sarcocystis* spp. microcysts. The number of microcysts detected was recorded.

RESULTS AND DISCUSSION

Among 200 samples which were analysed, 78.5% were positive for at least one parasite microcyst. In these samples, *T. gondii* and *Sarcocystis* spp. tissue cysts were detected as 2.5% and 56.5%, respectively; however, *T. gondii+Sarcocystis* spp. were found together in 19.5% of the samples. At least 62% of the beef, 68% of the sausage, 90% of the meatballs, 94% of the lamb and 90% of the sausage samples were infected with tissue cysts belonging to at least one species (Table 1). *Sarcocystis* spp. and *T. gondii* tissue cysts were detected in 76% and 22% of the samples, respectively.

Table 1. *Parasite tissue cysts and their rates detected in beef meat, lamb meat, sausage and butcher's meatballs*

Sample	***T. gondii* (only)**		***Sarcocystis* spp. (only)**		***T. gondii*+ *Sarcocystis* spp.**		**Negative**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Lamb meat	0	0	35	17.5	12	6	3	1.5
Beef meat	1	0.5	28	14	2	1	19	9.5
Sausage	4	2	20	10	10	5	16	8
Butcher's meatballs	0	0	30	15	15	7.5	5	2.5
Total	5	2.5	113	56.5	39	19.5	43	21.5

Toxoplasma gondii tissue cysts were detected 24% (12/50) and *Sarcocystis* tissue cysts 94% in lamb meat samples. These rates were 6% and 60% in beef, 28% and 60% in sausage samples, 30% and 90% in butcher meatball samples, respectively (Fig. 1).

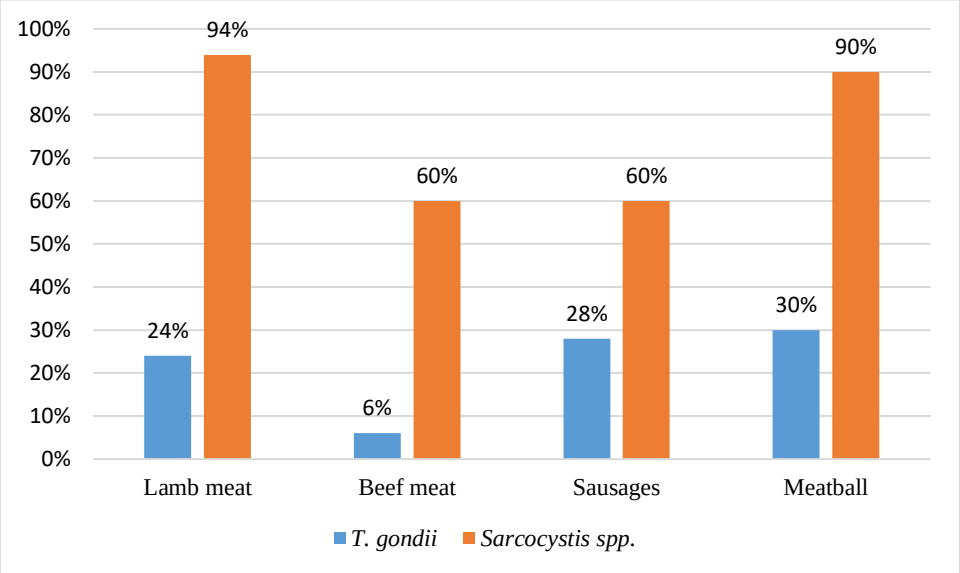

Fig. 1. *T. gondii and/or Sarcocystis* spp. *tissue cyst in beef meat, lamb meat, sausages and butcher meatball*

Ten lamella areas were counted from each of the samples analysed. Sarcocystis tissue cysts were detected in at least one and at most 72 areas in lamb meat, in at least one, and at most 15 areas in beef meat, in at least one and at most 102 areas in butcher's meatballs, in at least one and at most 38 areas in sausage. For *Toxoplasma gondii*, these ratios were 1 to 8 in lamb meat, 1 to 2 in beef meat, 1 to 14 in butcher meatballs and 1 to 9 in sausage (Fig. 2).

Fig. 2. *A: Sarcocystis* spp. *Microcyst; B: Toxoplasma gondii tissue cyst in lamb meat*

It was reported in a study conducted in Tekirdağ that *T. gondii* was not detected in raw red meat (sheep, goat and beef) and meat products (butcher meatballs, Tekirdağ meatballs, hand-chopped meatballs) examined by nested-PCR [12]. Another previous study conducted in Türkiye reported that parasite DNA was detected in 6% of cattle muscles, 20% of sheep muscles and 19% of fermented sausage samples [13]. It was determined that *T. gondii* tissue cysts were detected in sheep meat collected in Ankara and Kırıkkale regions by percoll gradient method at a rate of 20.8% and 22.4%, respectively, with an average of 21.2% [11]. In a study conducted in retail meat samples in Scotland, *T. gondii* DNA was detected at a rate of 6.9% in lamb meat samples, while all beef meat samples were negative [14]. In a study conducted in Iran, this rate was 4% in beef, 14% in mutton, and 0% in meat products (sausage, hamburger, salami) [15]. It was reported in Tunisia that 47.5% *T. gondii* DNA was detected in sausage samples by molecular analysis [16]. In this study, 22% *T. gondii* tissue cysts were detected in the samples examined. *T. gondii* tissue cysts were found in 3 (6%) of beef samples, 12 (24%) of lamb samples and 14 (28%) of sausage samples. Our study was the most common study in which *T. gondii was* detected in sausage samples when compared with the studies conducted in different regions of Türkiye and the world. The rate of *T. gondii* in lamb meat samples was similar to the previous studies in Türkiye, except for the one conducted in Tekirdag; however, such rate was higher than the studies conducted in different countries of the world. In beef samples, similar results were obtained to the studies conducted both in Türkiye and in the world. It was considered that the determination of different rates in some studies, especially in sheep meat may be related to the number of samples examined, the different examination techniques used, the feeding and care conditions of the sampled animals, the different control mechanisms of slaughter, production and sales places. In all studies, the rate of *T. gondii* in lamb meat was found higher than beef meat. This once again revealed that *T. gondii* may develop better in sheep.

In an Iranian investigation using digestive method to examine samples of hamburgers, sausages, and cocktails, 80% of the samples tested positive for Sarcocystis [17]. In another study, it was reported that 94.4% *Sarcocystis* bradyzoides were observed in beef and beef burger samples by peptic digestion method [18]. In Egypt, *Sarcocystis* spp. bradyzoides were detected in 58.57% of frozen beef, beef burger, sausage, lunch meat,

meatballs, minced meat and bacon samples imported from Brazil [19]. In this study, *Sarcocystis* microcystis were detected in 76% of beef, lamb, butcher's meatballs and sausage samples by percoll gradient centrifugation method.

Sarcocystis spp. tissue cysts were found in 60% of the sausage samples examined in our study. Sarcocystis rate was determined as 83.33% in sausage samples obtained from the markets of a factory in Iran by trypsin digestion technique [17]. In a study conducted in Ahvaz region of Iran, 8% *Sarcocystis* spp. was detected in sausages by microscopic examination [20]. In Egypt, the rate of Sarcocystis detected in sausages was 45% [19]. In a study conducted in Kars province in Türkiye, it was reported that *Sarcocystis* bradizoid was not detected in fermented sausage samples by microscopic examination [21], while in a study in Van, it was reported that 73.3% of *Sarcocystis* spp. microcysts were detected microscopically in sausages [22]. In Tunisia, 52.5% *Sarcocystis* spp. DNA was detected in local Merquez sausages in molecular analysis [16]. It is thought that the reason for the detection of *Sarcocystis* at different rates in the studies may be due to the methods used during the production of sausages, the nutritional conditions of the animals whose meat is used (pasture or intensive breeding) and therefore the different rates of *Sarcocystis* sporocysts.

It was reported in a study conducted in Iran that 93.3% of beef samples showed at least one *Sarcocystis* bradyzoid [18]. In studies conducted in Egypt, it was reported that between 6% and 90% Sarcocystis was detected microscopically in beef [19, 23]. It was reported that *Sarcocystis* spp. was found in 18 of 62 samples examined in the microscopic examination of beef for retail sale in China [24]. In a previous study conducted in Van province in Türkiye, *Sarcocystis* spp. was detected in 73.1% of beef [22]. In our study, 60% of *Sarcocystis* tissue cysts were found in beef. This rate is lower than the rates found in Egypt and Iran.

In a study conducted in Egypt, 55% *Sarcocystis* was found microscopically in meatballs [19]. In Iran, it was reported that *Sarcocystis* was detected in hamburgers at a rate of 20-95.5% in microscopic examination [4, 17, 18, 20, 25]. In a study conducted in Türkiye, 2% Sarcocystis bradizoid was detected in meatballs by microscopic examination [21]. In another study conducted in Turkey, 28% *Sarcocystis* spp. microcysts were detected in minced meat beef in Van province [26]. In our study, Sarcocystis rate in butcher meatballs was determined as 90%. The fact that some Sarcocystis species are zoonotic and the high rate of *Sarcocystis* detected reveals that meat control should be done better to protect human health.

Studies on the determination of *Sarcocystis* species in lamb meat are generally based on taking various tissues of lambs slaughtered in slaughterhouses and examining them by various methods. Studies to determine the prevalence of *Sarcocystis* species in lamb meat offered for sale in the market are limited. In Van, 51.2% *Sarcocystis* spp. microcysts were found in lamb meat offered to the market [22]. In a previous study conducted in Kırıkkale, Sarcocystis microcysts were detected 91% in lamb meat offered for sale using Percoll gradient method [27]. In this study, the rate of *Sarcocystis* spp. in lamb meat was 94%. The fact that *Sarcocystis* spp. were found at similar rates in these two studies conducted in the same region with an interval of approximately 10 years shows that the control of these parasites in the region is not at an adequate level. The determination of different rates in the studies conducted in Van and Kırıkkale may be associated with different diagnostic techniques used, the different climatic conditions of the provinces and the different feeding conditions of the animals sampled.

In the diagnosis of *Sarcocystis* spp. and *T. gondii* tissue cysts, digestive techniques such as pepsin etc. are generally used. The number of studies using Percoll gradient method is limited. This technique was used in a study conducted in Kırıkkale for the detection of *Sarcocystis* tissue cysts in sheep meat and microcysts were detected in 91% of the samples examined in this study [27]. In another study using the same method, *T. gondii* tissue cysts were found in 21.2% of sheep meat [13]. In a study conducted on buffalo meat, the rate of *T. gondii was* determined as 15% with the same method [28]. In this study, 62% of beef, 68% of sausage samples, 90% of butcher's meatballs, 94% of lamb meat analysed through Percoll gradient method were found infected with tissue cysts of at least one species (*Sarcocystis* spp. or *T. gondii*).

CONCLUSION

Toxoplasma gondii and *Sarcocystis* spp. tissue cysts were found in meat and meat products sold in Ankara and Kırıkkale. Since *Toxoplasma gondii* and some Sarcocystis species are zoonotic, it is very important for public health that meat and meat products are consumed after being well cooked. Furthermore, consumption of raw meatballs as a cultural dietary habit in Türkiye and insufficient attention to the cleanliness of knives and boards used in the slaughter of meat paves the way for *T. gondii* and *Sarcococytis* spp. species with zoonotic properties as well as other meat-borne infections. Therefore, taking the necessary hygiene measures is of great importance to reduce the risk of transmission of these parasites to humans. Necessary control and prevention measures should be taken to reduce the rate of *Sarcocystis* spp. and *T. gondii* tissue cysts in meat and meat products, and to prevent slaughter animals from being infected with the agent.

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: N.N.O., S.G., C.T., Design: N.N.O., S.G., C.T., Data Collection or Processing: N.N.O., S.G., C.T., Analysis or Interpretation: N.N.O., S.G., C.T., Literature Search: N.N.O., S.G., C.T., Writing: S.G.

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