Sarcocystis spp. prevalence in bovine minced meat: a histological and molecular study

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Abstract

Sarcosporidiosis is caused by ingestion of contaminated raw or undercooked bovine meat and, although considered a minor zoonosis, it can represent a threath for immunocompromised people. Aim of this study was to determine the prevalence of *Sarcocystis* spp. in bovine minced meat intended for raw consumption collected from butcher shops and retail stores in Turin's province (Piedmont region, Northwest Italy). Twenty-five samples were examined in parallel by histology and polymerase chain reaction (PCR). The prevalence of infestation of *Sarcocystis* spp. resulted to be 64% [confidence interval (CI) 95% 42-82] and 88% (CI 95% 69-97) respectively by histology and PCR. In detail, the prevalence resulted 80% for *S. cruzi* (CI 95% 59-93), 68% for *S. hominis* (CI 95% 46-85) and 4% for *S. hirsuta* (CI 95% 0.10-20). The high prevalence of *S. hominis* highlights that sarcosporidiosis may constitute a public health problem in Italy, particularly in regions like Piedmont, that has traditional dishes prepared from raw or undercooked bovine meat.

Introduction

The genus *Sarcocystis* includes more than 100 species with worldwide distribution. They are protozoan coccidian parasites belonging to the phylum *Apicomplexa*. They complete the life cycle in specific intermediate and definitive hosts or within closely related host species (Fayer, 2004).

Cattle can act as an intermediate host for *Sarcocystis cruzi* (Syn. *bovicanis*), *Sarcocystis hirsuta* (Syn. *bovifelis*) and *Sarcocystis hominis* (Syn. *bovihominis*), with canids, felids and primates, respectively, as definitive hosts (Dubey and Lindsay, 2006). The prevalence of *Sarcocystis* spp. in adult bovine muscle is close to 100% in most regions of the world where it has been studied (Vangeel *et al*., 2007). However, attention needs to be paid to the limited number of animals used in some surveys and the different analytical methods employed, that could not always differentiate the species (EFSA, 2010).

Recent studies carried out in Italy at slaughterhouses revealed, respectively, a prevalence of 96% (Bucca *et al*., 2011), above 80% (Domenis *et al*., 2011) and 91% (Chiesa *et al*., 2013).

Humans can host the intestinal phase of *S. hominis*, acquiring the infection through consumption of raw or undercooked infested bovine meat. Symptoms in humans are usually mild or in many cases absent and many authors considered the intestinal sarcocystosis as a minor zoonosis. Human sarcocystosis is not a notifiable disease in the EU Member States. In literature only a few articles on human intestinal sarcocystosis in Europe are available and most data were collected more than 15 years ago (Dubey *et al*., 1989; Fayer, 2004). Human intestinal sarcocystosis is not uncommon in Europe and prevalence data ranges from of 1.6% to 10.4% have been reported (Dubey *et al*., 1989).

Sarcocystosis is generally considered non-pathogenic for cattle. In the *post-mortem* examination the diagnosis can only regard few cases of animals showing gross lesions consisting of green to pale yellow areas up to 15 cm long, with consequent condemnation and economic losses (Dubey and Lindsay, 2006). The corresponding microscopical lesion is eosinophilic myositis, which is traditionally connected to the presence of *Sarcocystis* spp. (Vangeel *et al*., 2013).

In most cases, the muscular cysts referred to *Sarcocystis* can only be observed by light microscopy. However, definitive identification of *Sarcocystis* to the species level requires electron microscopy or molecular detection methods. Only a few studies made on retail bovine meat are present in literature (Pritt *et al*., 2008; Ghisleni *et al*., 2006; Jahed Khaniki and Kia, 2006; Vangeel *et al*., 2007). In some European countries there is a traditional cuisine characterised by the consumption of raw or undercooked bovine minced meat. In Italy, this consumption is particularly common in Piedmont. To our knowledge, in Italy no data about *Sarcocystis* spp. prevalence in raw bovine minced meat are available.

The aim of this work was to determine the prevalence of *Sarcocystis* spp., with special attention to *S. hominis*, in raw bovine minced meat, collected from butcher shops and retail stores in Turin's province (Piedmont region, Northwest Italy), as a potential source of *Sarcocystis* infection for consumers.

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Materials and Methods

Sample collection

Twenty-five bovine minced meat samples, representing 25 different animals, were collected from 13 retail stores (from five different store distribution chains) and 12 butcher shops located in Turin's province. The sampling was simple and randomly taken. We collected samples from 25 different stores, on different days, over a period of time long enough to be sure not to collect again the same animals. The samples were both from autochthonous cattle, *i.e.* Piedmontese breed, and from French breeds, such as Blonde d'Aquitaine breed. Because the sample size was small, we specified the confidence intervals (CIs) to allow the reader to correctly evaluate the data presented in our work. Each sample was split into two parts, one for histological esamination and one for polymerase chain reaction (PCR).

Histology

Each sample intended for histology was then subdivided into six parts, *i.e.* six fragments of minced meat were collected from each sample. To increase the representativeness of sample, each fragment was randomly taken. These six parts were fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding. Sections of 4±2 µm were stained with hematoxylin and eosin (HE) and carefully observed for the presence of *Sarcocystis* spp. cysts, at increasing magnification (x10, x20, x40). The sarcocysts appear as basophilic bodies, round or elongated in shape depending on the cutting plane, bordered by a radial fairly thick wall. Samples were considered positive

when one or more cysts were detected in at least one section of the sample. Thin-walled sarcocysts are considered consistent with *S. cruzi* (Figure 1), while thick-walled sarcocysts (Figure 2) could represent either *S. hominis* or *S. hirsuta*. Sarcocysts wall thickness was measured using a digital micro-imaging Leica DMD108 (Leica Microsystems GmbH, Wetzlar, Germany). In total, one hundred fifty sections were examined.

Molecular typing

The portion for PCR was randomly collected from the samples. Molecular typing was performed on a portion of 25-mg tissue with a differential PCR described by Vangeel *et al.* (2007), applied using a modified reverse primer labeled with Hex fluorescent dye at the 5 (Domenis *et al*., 2011). This PCR amplifies a region of the 18S rRNA gene generating amplicons of different lengths depending on the *Sarcocystis* species: 164 bp for *S. hominis*, 172 bp for *S. cruzi*, and 186 bp for *S. hirsuta*. The PCR products were submitted to capillary electrophoresis on an ABI 3130 Genetic Analyser and the size was determined by GeneMapper software analysis.

Additional histologic sections

Following PCR, three additional histologic sections were obtained from the six cases for which histological examination had not previously identified sarcocysts but in which PCR had detected parasite DNA (Pritt *et al*., 2008).

Statistical analysis

Prevalence of infestation for *Sarcocystis* spp. to a regional level was calculated by STATA 11, with relative CIs.

Results

Histology

Examination of histological sections revealed the presence of sarcocysts in 64% (16/25) of the samples, *i.e.* histologically the prevalence of infestation of *Sarcocystis* spp. was 64% (CI 95% 42-82). Specifically, out of the 16 positive samples, only 1 section was positive in 3 samples, 2 sections in 7 samples, 3 sections in 1 sample, 4 sections in 1 sample, 5 sections in 1 sample, all 6 sections in 3 samples. : In 48% (12/25) of samples both thin-walled cysts and thick-walled cysts were observed, while in 16% (4/25) of samples only thin-walled cysts were observed. No pathological changes were observed in muscular fibres or surround interstitium.

Molecular typing

All three species of *Sarcocystis* affecting cattle were identified by molecular typing. Of the samples, 88% (22/25) resulted positive for the presence of at least one *Sarcocystis* spp., *i.e.* by PCR the prevalence was 88% (CI 95% 69-97) for *Sarcocystis* spp. Specifically, the prevalence was 80% for *S. cruzi* (CI 95% 59-93), 68% for *S. hominis* (CI 95% 46-85) and 4% for *S. hirsuta* (CI 95% 0,10-20). 12% (3/25) of the samples resulted negative. Of 25 samples, 15 (60%) revealed the presence of two or more species. Specifically, 56% (14/25) of the samples showed a co-infestation with *S. cruzi* and *S. hominis*, 20% (5/25) infestation with *S. cruzi*, 8% (2/25) infestation with *S. hominis*, 4% (1/25) co-infestation with *S. hominis-S. cruzi-S. hirsuta* (Figure 3).

Additional histologic sections

Sarcocysts were observed in two out of six samples.

Discussion

Bovine meat can be infested by three species of *Sarcocystis*: *S. cruzi*, *S. hirsuta* and *S. hominis*. Only *S. hominis* is considered potentially zoonotic. Sarcosporidiosis is caused by ingestion of contaminated raw or undercooked bovine meat and, although considered a minor zoonosis, it can represent a threath for immunocompromised people.

The histological differentiation between bovine thin-walled sarcocysts (*S. cruzi*) from thick-walled sarcocysts (*S. hirsuta* and *S. hominis*) is simple, whereas the distinction between *S. hirsuta* and *S. hominis* is difficult (Ghisleni *et al*., 2006). For this reason, species identification requires the application of electron microscopy or molecular methods.

Prevalence data in literature are generally referred to samples collected at the slaughterhouse. A recent study carried out in Piedmont (Domenis *et al*., 2011) showed a prevalence in cattle above 80%; in another recent study (Chiesa *et al*., 2013) the authors found a prevalence of *Sarcocystis* spp. in cattle of 91%. In Sicily (Southern Italy) Sarcocysts were detected in the 96% of animals (Bucca *et al*., 2011). Only few prevalence studies are referred to meat-based preparations or products. In Belgium, *S. hominis* was detected in 97.4% of the minced beef samples (Vangeel *et al*., 2007).

The diagnostic protocol applied in our study proved to be both useful and efficient. Although histological detection of sarcocysts in some cases resulted difficult due to matrix, sarcocysts wall thickness can be evaluated. It is important to note that in all cases resulted positive by histology the histological differentiation between thin-walled sarcocysts from thick-walled sarcocysts was correctly performed, because there was correspondence between tipology of wall observed by histology and species identificated by PCR. Domenis *et al*. (2011) showed that *S. hirsuta* in Piedmont is nearly absent and suggested that simple histological observation of sarcocysts wall thickness could be a useful tool to detect the presence of *S. hominis* in cattle, without the need for more complex and expensive techniques, such as electron microscopy and molecular

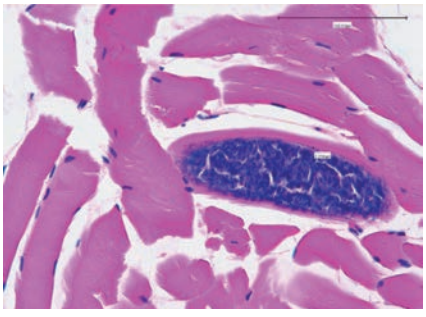

Figure 1. One thin-walled sarcocyst. Hematoxylin and eosin, magnification x40.

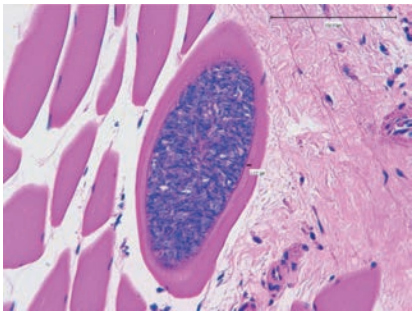

Figure 2. One thick-walled sarcocyst. Hematoxylin and eosin, magnification x40.

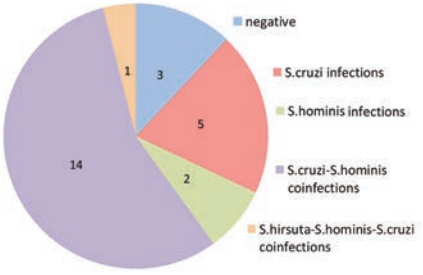

Figure 3. Results of analyses on the samples related to the presence of individual *Sarcocystis* species.

methods. In their work *S. hirsuta* was detected in 7/384 samples (prevalence 1.8%, CI 0.7-3.7) and in the present paper in 1/25 samples (prevalence 4%, CI 95% 0.10-20), the overlapping of the two CIs points out the according of the results.

By examining six sections for each sample, the sensitivity of histology compared with PCR (with PCR as gold standard) was 72.7% (CI 95% 49.8%-89.3%), the specificity was 100% (CI 95% 29.2%-100%). For six samples, nine sections for sample were histologically examined and, on the basis of the results obtained for these six samples, *i.e.* additional two out of six samples resulted histologically positive, we can observe that the examination of additional tissue sections may increase the detection capability for sarcocysts; this aspect related to diagnostic techniques could be deepened with a next work. Polymerase chain reaction represents an effective and rapid method for an unbiased identification of *Sarcocystis* spp and the application of capillary electrophoresis sizing to the differential PCR described by Vangeel *et al.* (2007) significantly improved the resolution of the molecular method (Domenis *et al*., 2011). Histology could be considered as less expensive even if time-consuming in comparison with PCR.

Conclusions

Our study revealed a relatively high prevalence of *S. hominis* (68%). Although several infection studies on human volunteers have been carried out and, even with what was considered a high infection dose, only mild symptoms were reported. The EFSA recommends to seek clarification of the relevance of *S. hominis* for public health (EFSA, 2010). Furthermore, although Sarcosporidiosis cannot be considered as a major zoonosis, data obtained in this study on the occurrence of sarcosporidiosis are relevant for human health, especially for the studied region, Piedmont, in which the consumption of raw or undercooked bovine meat is common.

References

Bucca M, Brianti E, Giuffrida A, Ziino G, Cicciari S, Panebianco A, 2011. Prevalence and distribution of Sarcocystis spp. cysts in several muscles of cattle slaughtered in Sicily, Southern Italy. Food Control 22:105-8.

Chiesa F, Muratore E, Dalmasso A, Civera T, 2013. A new molecular approach to assess the occurrence of Sarcocystis spp. in cattle and products thereof: preliminary data. Ital J Food Safety 2:e41.

Domenis L, Peletto S, Sacchi, Clementi E, Genchi M, Felisari L, Felisari C, Mo P, Modesto P, Zuccon F, Campanella C, Maurella C, Guidetto C, Acutis P, 2011. Detection of a morphogenetically novel Sarcocystis hominis-like in the context of a prevalence study in semi-intensively bred cattle in Italy. Parasitol Res 109:1677-87.

Dubey JP, Speer CA, Fayer R, 1989. Sarcocystis of animals and man. CRC Press, Inc., Boca Raton, FL, USA.

Dubey JP, Lindsay DS, 2006. Neosporosis, toxoplasmosis, and sarcocystosis in ruminants. Vet Clin Food Anim 22:645-71.

EFSA, 2010. Development of harmonised schemes for the monitoring and reporting of Sarcocystis in animals and foodstuffs in the European Union. Available from: http://www.efsa.europa.eu/it/supporting/doc/33e.pdf

Fayer R, 2004. Sarcocystis spp. in human infections. Clin Microbiol Rev 17:894-902.

Ghisleni G, Robba S, Germani O, Scanziani E, 2006. Identification and prevalence of Sarcocystis spp. cysts in bovine canned meat. Food Control 17:691-4.

Jahed Khaniki GR, Kia EB, 2006. Detection of Sarcocystis cysts from meat supplied for hamburger in Iran by histological method. J Med Sci 6:18-21.

Pritt B, Trainer T, Simmons-Arnold L, Evans M, Dunams D, Rosenthal BM, 2008. Detection of sarcocystis parasites in retail beef: a regional survey combining histological and genetic detection methods. J Food Protect 71:2144-7.

Vangeel L, Houf K, Chiers K, Vercruysse J, D'Herde K, Ducatelle R, 2007. Molecular-based identification of Sarcocystis hominis in Belgian minced beef. J Food Protect 70:1523-6.

Vangeel L, Houf K, Geldhof P, De Preter K, Vercruysse J, Ducatelle R, Chiers K, 2013. Different Sarcocystis spp. are present in bovine eosinophilic myositis. Vet Parasitol 197:543-8.