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 threat 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. bovilest) 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 slaughtermen located, 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 Kaniaki 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.

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 examination 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 haematoxylin and eosin (HE) and carefully 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...
revealed the presence of sarcocysts in 64% (10/25) 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-infection with S. cruzi and S. hominis, 20% (5/25) infestation with S. cruzi, 8% (2/25) infestation with S. hominis, 4% (1/25) co-infection 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 threat 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 identified 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.
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.

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