The Prevalence of Sarcocystis Affecting Slaughtered Cattle and Buffalo at Sirs-Elian Abattoir in Egypt

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ABSTRACT

The importance of Sarcocystis emerged from the public health point of view, as it causes zoonotic infection. This study aimed to investigate the prevalence of Sarcocystis in economically important animals (cattle and buffalo) to lower the zoonotic potential of this parasite. A total of 7435 slaughtered animals (3879 cattle and 3556 buffalo) were examined macroscopically and microscopically for the detection of Sarcocystis followed by molecular detection and identification using PCR and Sequencing of 18S rRNA gene. The overall infection of macroscopic Sarcocystis was a higher prevalence in females 62.73% than in males (0.73%). In buffalo, the higher prevalence of macroscopic Sarcocystis observed in esophagus 46.82% tongue 9.3%, forequarter 3.9%, for buffalo female and male. Although the overall prevalence of microscopic cyst was 87%, the highest prevalence was in heart 63% (88% and 38%), esophagus 56% (72% and 40%), forequarter 55% (64% and 46%), tongue 41% (36% and 46%), diaphragm 32% (26% and 38%), hindquarter 27% (30% and 24%) in cattle and buffalo. Furthermore, the molecular identification showed that isolates Sarcocystis were S. cruzi, and S. hominis in cattle and buffalo, and S. Levinei and S. sinensis in buffalo comparing with published data on GenBank. The present study is the first report identified S. hominis infection in Egyptian buffaloes in Menoufia Governorate and the second record of identifying S. cruzi in Egyptian buffaloes. Using PCR is a more sensitive method in the detection of different Sarcocystis spp. In Egypt, a greater hygienic application is required.

1.INTRODUCTION

As the Sarcocystis is one of the obligatory lifecycle cysts-forming coccidian parasite composed of more than 200 species (Frenkel and Smith, 2003) and has ubiquitous global distribution, as the meat is one of the potential sources of the infection with this parasite giving it an importance of the concepts of food safety. There are some factors controlling the prevalence of Sarcocystis at which its existence varies which are: the ecological conditions (Savini et al., 1992), hygiene in different animal localities, the age groups of the examined animals (El-Kelesh, 2011), and the extent of a final host in contact with the intermediate one as canids the definitive host for microscopic cyst, while the felids is a definitive host for the macroscopic cyst.

The Problems as meat-borne parasites are becoming more of concern for many reasons as increasing imports of food some of which originate without modern sanitary facilities and inspection systems may introduce parasites, popularity of raw and/or lightly
cooked foods may increase exposure to parasites (CDC, 2000). Sarcocystosis caused by different Sarcocyst species in man and many species of animals. Sarcocyst species, generally alternating between an herbivorous intermediate host and a carnivorous definitive host as they obligate two-host parasites (Pena et al., 2001; Nourollahi-Fard et al., 2015). The mature Sarcocyst of each species can range in size from microscopic to visible by the naked eye and so it’s shapes vary from spindle to globular. These features vary with the age of Sarcocyst and the host cell type (Yang et al., 2002; Pritt et al., 2008). The methods of diagnosis of Sarcocyst infection in different animals are very difficult as the clinical signs are nonspecific (Dubey et al., 2015). It is useful to identify Sarcocyst species by the variable regions of the 18S rRNA gene as it provides suitable targets for the identification and characterization of different species even among the same genus. In Egypt, cattle and water buffalo are also raised as strategic wealth animals. The aim of this study is to investigate the prevalence of Sarcocystis infection in these two economically important animals slaughtered at the Sirs-Elian abattoir in Menoufia Governorate, Egypt, using macroscopic and microscopic examination, as well as molecular detection and identification using conventional PCR and sequencing of 18S rRNA gene to lower the zoonotic potential of this parasite.

2.MATERIALS AND METHODS
2.1. Samples Collection
A total of 7435 slaughtered animals of different ages and sex represented by 3879 cattle and 3556 buffalo were examined among the routine daily post-mortem examination according to the legal requirements of Egyptian abattoirs. The samples were collected during the period between the end of December 2014 to the end of December 2018 at sirs-Elian abattoir, Menoufia Governorate, Egypt, for detection of macroscopic Sarcocystis in muscular tissues of esophagus, heart, tongue, diaphragm, fore quarter muscles, and hind quarter muscles which carefully inspected by naked eye and palpate for the presence of macroscopic sarcocystis. The results were recorded after grouping the animals to cattle (male and female) and water buffaloes (Male and female).

From the apparently 100 negative animals, 50 cattle (25 male and 25 female) and 50 water buffalo (25 male and 25 female), 200 gm tissue samples/each animal was collected for pepsin digestion technique. Furthermore, the samples were collected for molecular detection and identification of sarcocystis as the following: Six different tissue samples (esophagus, tongue, heart, diaphragm, forequarter, and hindquarter) were collected from each four slaughtered animals of both sex which apparently positive by naked eye inspection, four slaughtered animals of both sex which apparently negative by naked eye inspection, four slaughtered animals of both sex which apparently positive by pepsin digestion technique and four slaughtered animals of both sex which apparently negative pepsin digestion technique. All collected samples were stored at -20°C until DNA extraction. All procedures were acceptable by the local ethical committee of Animal use, Faculty of Veterinary Medicine, Alexandria university, Egypt.

2.2. Macroscopic examination
The macroscopic Sarcocystis were detected by cut muscular tissues samples into fine pieces and examined by naked eye, and palpation according to (Soulsby, 1982; Gracy, 1986). If Sarcocystis was found the sample considered positive macroscopically if it proceeds to microscopic examination by digestion technique.

2.3. Pepsin digestion and microscopic examination
The digestion process was carried out by slicing the sample (50 gm) into smaller pieces to increase its surface area, then blending it in a 125 ml digestion solution (Pepsin and saline) and adjusting the PH to 2 with strong HCL. The mixture was incubated in a water bath for 15 minutes, after which the PH was adjusted again, and the beaker was covered and incubated at 37°C until digestion was complete.

The digestion process was carried out by slicing the sample (50 gm) into smaller pieces to increase its surface area then blended it in 125 ml digestion
solution (Pepsin and saline), and adjusted the PH. The mixture was incubated in a water bath for 15 min then adjusts PH again, then covered the beaker and incubated at 37°C until digestion completed in not more than 24 hrs. The contents of beaker were carefully poured into the tray through a sieve. The residue rinsed with 250 ml saline then digest. Rinsed contents of the sieve were examined for macroscopic cysts. The content of the tray transferred to a sedimentation cone. The undigested parasites were shifted to a petri dish than after 1 h, 50 ml of sediment were collected in the beaker and diluted with saline than examined macroscopically for parasites. The sediment centrifuged at 1500 rpm for 10 min, the supernatant discarded, and the sediment stained by Giemsa stain and examined microscopically according to (Latif et al., 1999).

2.4. Molecular identification

2.4.1. DNA extraction

Genomic DNA was extracted from tissue samples using DNA extraction kit (iNtRON Biotechnology, Korea) according to manufacture instruction. The extracted DNA quality was assessed by running on 2% agarose gel stained with ethidium bromide. The electrophoresis gel was examined in the UV transilluminator, and bands were visualized and photographed using a gel documentation system (Gel Doc. Alpha-chem. Imager, USA).

2.4.2. Gene amplification and purification

The partial sequence of 18S ribosomal RNA (18S rRNA) gene, was amplified by using the 18S rRNA gene of Sarcocystis genus spp. primer (18S9LF: GGATAACCTGGTAATTCTATG and 18S1HR: GGCAAATGCTTTCGCAGTAG). This primer only amplifies approx. 900 bp fragment and does not amplify host DNA (Li et al., 2002). The PCR was performed in 50 μl reaction volume, containing 25 μl master mix, 5 μl genomic DNA, 1 μl of each primer, 18 μl dH2O which finally added. The final reaction mixture was placed in the thermal cycler (Technee, TC-3000, USA). The PCR program was carried out by initial denaturation at 95 °C for 5 min followed by 35 cycles of 94 °C for 1 min for DNA denaturation, annealing temperature 56 °C for 1 min, extension at 72 °C for 1 min and final extension at 72°C for 10 min. PCR product of each sample and 100 bp DNA ladder (GeneDireX, Taiwan) were loaded in 2% agarose gel stained with ethidium bromide. The electrophoresis gel was visualized and photographed using gel documentation system (Gel Doc. Alpha-chem. Imager, USA). The PCR products of 18S rRNA gene of Sarcocystis genus spp. Primer from different tissue samples were purified using MEGAquick-spin™ total fragment DNA purification kit (Intron Biotechnology, Korea) according to manufacturer's instruction.

2.4.3. DNA Sequencing and Data Analysis.

The sequencing was performed for 12 purified PCR products of different tissue samples, of both cattle and buffalo, which were positive diagnostic band with the 18S rRNA gene of Sarcocystis genus spp. primer. The purified PCR products were sequenced in an ABI 3730XL DNA sequencer (Genetic Analyzer, Applied Biosystem, Hitachi, Japan) by using the same primer (Forward direction 5’-3’) used in PCR. The sequences results were analysed using Chromas 1.45 (http://technelysium.com.au/wp/). The comparison of obtained sequences with those previously published in the NCBI was performed using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) based on highly similar sequences available on GenBank. The sequences were aligned using CLUSTALW version 1.8 (Thompson et al., 1994).

2.4.4. Statistical analysis

The statistical analysis was carried out using Ch² test to examine the detection of the significance of the prevalence of Sarcocystis among animal species (cattle and buffalo), sex, and different examined tissues samples. The analysis was made according to (SAS, 2004) Version -5.
3. RESULTS

The result of examination of 7435 slaughtered animals (cattle and buffalo) revealed that the overall infection of macroscopic cyst in female 62.73% of a higher prevalence than in male (0.73%) as macroscopic cyst was not found in cattle while in buffaloes (63.39%, and 3.64%) in female and male (Table 1). The higher prevalence of macroscopic Sarcocystis observed in buffalo’s tissues as follow: esophagus 46.82% (62.80%, and 3.64%), tongue 9.3% (12.56%, and 0.52%), diaphragm 0.39% (0.3%, and 0.62%), forequarter 3.9% (5.09%, and 0.73%), and hindquarter 0.92% (1.27%, and 0%) for female and male, and could not detected in the examined heart (Table 2 and Fig. 1).

Table 1. Overall prevalence of macrocysts of sarcocystis.

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>Infected carcasses</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Cattle</td>
<td>3879</td>
<td>nil</td>
<td>3852</td>
<td>nil</td>
</tr>
<tr>
<td>Buffalo</td>
<td>3556</td>
<td>1680</td>
<td>961</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>7435</td>
<td>1680</td>
<td>4813</td>
<td>35</td>
</tr>
</tbody>
</table>

Chi² = 10.25**, ** = Significant at (P < 0.01), Nil = negative

Table 2. Prevalence of macroscopic sarcocyst in water buffalo’s different tissues.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total</th>
<th>+ve %</th>
<th>Esophagus</th>
<th>Heart</th>
<th>Diaphragm</th>
<th>Tongue</th>
<th>Fq</th>
<th>Hq</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Female</td>
<td>2595</td>
<td>1645</td>
<td>1630</td>
<td>62.8</td>
<td>nil</td>
<td>0</td>
<td>8</td>
<td>0.3</td>
</tr>
<tr>
<td>Male</td>
<td>961</td>
<td>35</td>
<td>35</td>
<td>3.64</td>
<td>nil</td>
<td>0</td>
<td>6</td>
<td>0.62</td>
</tr>
<tr>
<td>Total</td>
<td>3556</td>
<td>1680</td>
<td>1665</td>
<td>46.82</td>
<td>nil</td>
<td>0</td>
<td>14</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Chi² = 14.45**, ** = Significant at (P < 0.01), Nil = negative

While the overall prevalence of microscopic cyst 87% (88%, and 86%) the higher prevalence was in heart 63% (88%, and 38%), esophagus 56% (72%, and 40%), forequarter 55% (64%, and 46%), tongue 41% (36%, and 46%), diaphragm 32% (26%, and 38%), and hindquarter 27% (30%, and 24%) in cattle and buffaloes (Table 3 and 4). Additionally, The prevalence of Sarcocystis infection in the examined beef samples by conventional PCR method using 18S rRNA gene of sarcocystis genus spp. primer showed that, there is a significant difference (P < 0.01) of the incidences of Sarcocyst infection in examined beef samples. Where the higher incidences observed in heart, hind quarter, esophagus (93.75%, 87.50% and 87.50%) respectively, and the lower incidences observed in diaphragm 68.70% (Table 5).

Table 3. Prevalence of microscopic sarcocyst in water buffalo’s different tissues.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total no.</th>
<th>+ve %</th>
<th>Esophagus</th>
<th>Heart</th>
<th>Diaphragm</th>
<th>Tongue</th>
<th>Fq</th>
<th>Hq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>25</td>
<td>20</td>
<td>80</td>
<td>8</td>
<td>32</td>
<td>10</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>23</td>
<td>92</td>
<td>12</td>
<td>48</td>
<td>9</td>
<td>36</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>43</td>
<td>86</td>
<td>20</td>
<td>40</td>
<td>19</td>
<td>38</td>
<td>19</td>
</tr>
</tbody>
</table>

Fq = Forequarter and Hq= Hindquarter.

![Fig. 1: Macroscopic Sarcocystis in different buffalo's tissues:](image)
Table 4. Prevalence of microscopic sarcocyst in cattle’s different tissues.

<table>
<thead>
<tr>
<th>Sex</th>
<th>No.</th>
<th>+ve</th>
<th>%</th>
<th>Esophagus</th>
<th>Heart</th>
<th>Diaphragm</th>
<th>Tongue</th>
<th>Fq</th>
<th>Hq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>25</td>
<td>23</td>
<td>92</td>
<td>19</td>
<td>76</td>
<td>23</td>
<td>100</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>21</td>
<td>84</td>
<td>17</td>
<td>68</td>
<td>21</td>
<td>84</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>44</td>
<td>88</td>
<td>36</td>
<td>72</td>
<td>44</td>
<td>88</td>
<td>13</td>
<td>26</td>
</tr>
</tbody>
</table>

Fq = Forequarter and Hq = Hindquarter

Table 5. Prevalence of Sarcocystis infection in the examined beef samples by conventional PCR method.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>No</th>
<th>%</th>
<th>No</th>
<th>%</th>
<th>No</th>
<th>%</th>
<th>No</th>
<th>%</th>
<th>No</th>
<th>%</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>Male (4)</td>
<td>3</td>
<td>75</td>
<td>3</td>
<td>75</td>
<td>4</td>
<td>100</td>
<td>3</td>
<td>75</td>
<td>4</td>
<td>100</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Female (4)</td>
<td>4</td>
<td>100</td>
<td>4</td>
<td>100</td>
<td>2</td>
<td>50</td>
<td>4</td>
<td>100</td>
<td>2</td>
<td>50</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Buffalo</td>
<td>Male (4)</td>
<td>3</td>
<td>75</td>
<td>4</td>
<td>100</td>
<td>4</td>
<td>100</td>
<td>4</td>
<td>100</td>
<td>4</td>
<td>100</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Female (4)</td>
<td>4</td>
<td>100</td>
<td>4</td>
<td>100</td>
<td>1</td>
<td>25</td>
<td>2</td>
<td>50</td>
<td>3</td>
<td>75</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Total (16)</td>
<td></td>
<td>14</td>
<td>87.50</td>
<td>15</td>
<td>93.75</td>
<td>11</td>
<td>68.70%</td>
<td>13</td>
<td>81.25%</td>
<td>13</td>
<td>81.25%</td>
<td>14</td>
<td>87.50%</td>
</tr>
</tbody>
</table>

Chi² = 12.45**  ** = Significant at (P < 0.1)  Fq = Forequarter and Hq = Hindquarter

Figure 2 (a and b) shows the PCR products of 18S rRNA gene of sarcocystis genus spp. primer from different tissue samples of cattle and buffaloes. To identify Sarcocystis species, purified PCR products of 18S rRNA gene of Sarcocystis genus spp. primer were successfully sequenced in one direction using forward primer. The comparison, alignment and phylogenetic tree of cattle and buffalo obtained sequence with published data on GenBank using NCBI Blast program and Clustalw program, revealed that the obtained partial sequence of 18S rRNA gene have similarity with the corresponding sequence of S.cruzi with accession no. LC171828.1, LC171829.1 :99%, LC171827.1: 100% and MH129611.1: 99% (Fig. 3) and with sequence of s. hominis with accession no. JX679471.1 :93%, AF006471.1, AH006014.2 :94% and AF176945.1: 93% (Fig. 4).

Fig. 2. PCR product of 18S rRNA gene of sarcocystis genus spp. primer from examined different cattle (a) buffaloes (b) tissue samples, lane M 1500-bp ladder DNA marker and the other lanes represent the positive diagnostic bands of the 18S rRNA gene approx. 900bp in different tissue samples (T: tongue, E: esophagus; Hq: Hindquarter, Fq: Forequarter, H: Heart, D: Diaphragm).
Fig. 3. DNA sequence alignment of 18S ribosomal RNA gene in tissue samples compared with the published sequences of *S. cruzi* on GenBank (Accession No. LC171828.1, LC171829.1, LC171827.1 and MH129611.1), ordered according to ClustalW2Program. Cattle tissue samples No (1, 2, 3 and 4) and buffalo tissue samples No (5, 6 and 7).

In buffalo, the sequence analysis revealed that the partial sequences of the 18S rRNA gene were homology with the published data of *S. levinei* (accession no. KU247922.1, MG957189.1 and MN197852.1: 100%) and *S. sinensis* (accession no. JQ713823.1 and AF266960.1: 97%) as shown in supplementary fig. 1 (a and b). In the present study, the result indicates that using the PCR was more sensitive identification method than the other methods. Additionally, there was more than one Sarcocystis species circulating in cattle and buffaloes in Egypt.
Fig. 4. DNA sequence alignment of 18S ribosomal RNA gene in cattle and buffalo tissue samples (No. 8 and 9) compared with the published sequences of S. hominis on GenBank (Accession No. JX679471.1, AF006471.1, AH006014.2 and AF176945.1), ordered according ClustalW2Program.

4. DISCUSSION

Sarcocystis considers one of the major causes of economic losses of beef meet, also it may cause a health problem to consumers. In the present study prevalence of macrocysts of Sarcocystis among cattle and buffalo cleared that, the higher prevalence of macrocysts of Sarcocystis observed in female’s buffalo (63.39 %) followed by male’s buffalo (3.64 %) and there is no prevalence s of macrocysts of Sarcocystis in both sex in cattle (Table 1). Furthermore, the results showed that, the total infection rate in females (62.73%) was a higher prevalence than in males (0.73%). It is possible that the final hosts (dog and cat) are highly infected and permanently shed an abundant amount of sporulated oocysts and intermediate hosts are in persistent contact with the shaded infective oocysts, and sporocyst (Latif et al., 1999). No macroscopic cyst of sarcocyst was observed in cattle either males or females. Similarly, other studies reported that there was no macroscopic Sarcocystis cyst in examined cattle, although of microscopic Sarcocystis cysts prevalence in cattle was noticed in 100% (Nourollahi-Fard et al., 2009; Badawy et al., 2012; Hamidinejat, 2015; Nourollahi-Fard et al., 2015; El-Mokadem, 2016; Mirzaei and Rezaei, 2016; Yang and Dong, 2018). In general, the higher prevalence s of macroscopic Sarcocystis observed in a buffalo’s esophagus (46.82%), the lower prevalence s observed in tongue (9.30%) and fore quarter (3.90%), (Table 2). While the least prevalence s observed in the hind
quarter (0.92%) and diaphragm (0.39%) as, there is no Sarcocystis in the examined heart this agreed with Haddadzadeh et al., (2004) and Ghorbanpoor, (2007). The overall prevalence of macroscopic Sarcocystis among different tissue elucidate that the esophagus is the highest organ nearly the same result obtained by Abu-Elwafa et al., (2015) who reported 100% in esophagus and elevated rates in other tissues, also the result obtained by Ahmed et al., (2016) at a rate (76.26%). Previous studies indicated that Sarcocystis was recorded frequently in the esophagus as the esophagus may has the best conditions for the development of the parasite (Domenis et al., 2011; Savini et al., 1992; Taib et al., 2016). On the other hand, Dar et al., (2017) reported that tongue had the highest prevalence rate of infection. Additionally, Morsy et al., (2018) showed that the most infected buffalo's organ with macroscopic Sarcocystis was esophagus, followed by the diaphragm, and tongue, while heart was least infected. All these results give us strong evidence that the prevalence of Sarcocystis does not pursue a specific pattern in most of the affected organs. The results observed in table (3) cleared that, there is a significant difference (P<0.01) of the prevalence of microscopic sarcocyst in water buffalo at different tissue. The higher prevalence observed in tongue and fore quarter (46%) followed by esophagus (40%), diaphragm and heart (36%), and the lower prevalence observed in the hind quarter (24%). The higher prevalence of the sarcocyst microcyst in buffalo observed in female higher than its prevalence in male except in heart and fore quarter in male higher than the female. While the present results on the prevalence of microscopic sarcocyst in cattle at different tissues cleared that, the higher prevalence observed in heart with (88%), esophagus (72%), fore quarter (64%) and the lower prevalence observed in the hind quarter (30%) and the least prevalence observed in diaphragm (26%) (Table 4). Moreover, there is a significant difference (P<0.01) of the prevalence of microcyst Sarcocystis in cattle at different tissue as shown in table (4). Likewise, Aldemir and Güçli, (2004) revealed a higher prevalence of microscopic Sarcocystis in the oesophagus with 92%, followed by the heart with rates infestation of 84%.

The total prevalence of Sarcocystis infection by using the molecular identification techniques (PCR) in examined tissues was 68.75%, increasing by 18.75% from others both techniques. As it was 13.45% in cattle male, 14.6% in cattle female, 18.75% in buffalo male, and 22.40% in buffalo female. Another study also reported that the Sarcocystis infection rate in examined different cuts of imported frozen buffalo meat was 70.9 % by molecular methods while it was 23% and 20% by macroscopic and microscopic examination (Mousa et al., 2016). Molecular characterization techniques based on sequence analysis of the 18S rRNA gene, which used as genetic markers for species-specific differentiation of Sarcocystis spp. (Hamidinejat et al., 2014; Imre et al., 2019; Li et al., 2002; Murata et al., 2018). In this study, the alignment results of DNA sequences of the PCR product from different tissue samples revealed a mixed infection of slaughtered cattle and buffalo with S. cruzi with accession no. LC171828.1, LC171829.1, LC171827.1 and MH129611.1 and S. hominis with accession no. JX679471.1, AF006471.1, AH006014.2 and AF176945.1. Moreover, S. cruzi is highly prevalent in cattle worldwide, with ranged from 24 to 100%, as recognized in several studies, whereas a few of these studies made molecular identification (Jehle et al., 2009; El-Kady et al., 2018). As S. cruzi are not supposed to occur in water buffalo, but Jehle et al., (2009) decided that certain parasites are shared by cattle and water buffalo. Our study considers the second record of identify S. cruzi in Egyptian buffaloes in Menoufia Governorate after the first recorded case from Assiut Governorate, Egypt by Metwally et al., (2014), they confirmed the hypothesis that S. cruzi can use water buffalo as intermediate hosts. Furthermore, the present study is the first report identified S. hominis infection in Egyptian buffaloes in Menoufia Governorate. Whereas S. hominis is a zoonotic species can cause digestive turbulences such as gastrointestinal symptoms, vomiting, diarrhea (Abu-Elwafa et al., 2015). S. cruzi also, caused considerable morbidity and mortality in cattle (Woo and Shin, 2001).

A recent study reported that definite identification of S. hominis by multiplex PCR permits the estimated prevalence of this zoonotic Sarcocystis spp in the meat (Rubiola et al., 2020). Several studies successfully identify S. hominis in cattle in different parts of the world, such as in Vietnam, Argentina, and Nigeria (Jehle et al., 2009; Moré et al., 2011; Obijiake et al., 2013).

Additionally, the sequence analysis of 18S rRNA gene obtained from different tissue samples of buffalo was performed and compared with the reference sequences of Sarcocystis species in the GenBank, which identified that these sequences were homology with the published data of S. levinei (accession no. JQ713823.1 and AF266960.1) and S. sinensis.
(accession no. KU247922.1, MG957189.1 and MN197852.1). Gjerde et al., (2016) reported that differentiation of S. levinei and S. cruzi was possible by using 18S rRNA gene sequences. Therefore, the comparison of produced 18S rRNA gene sequences of S. levinei of water buffaloes with similar sequences deposited in GenBank suggested that S. levinei and S. cruzi was not firmly intermediate host specific but might infect cattle and water buffaloes, respectively. So, the present results indicated that Sarcocystis spp. are not host-specific.

5. CONCLUSION

The data presented in this study revealed a high prevalence of Sarcocystis infection among slaughtered cattle and buffalo. Different Sarcocystis spp were identified by sequencing of 18S rRNA gene, S. cruzi and S. hominis in both cattle, and buffalo, S. levinei and S. sinensis in buffaloes. Sarcocystis considers one of a major cause of economic losses of beef meat. We strongly recommend using the microscopical examination and molecular identification in routine examination for Sarcocystosis in Egypt slaughterhouse.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research article.

Authors Contribution

All authors contributed equally to carry out this work.

REFERENCES


