Research Article

TRADITIONAL AND MOLECULAR Cryptosporidium spp. RECOGNITION OF CATTLE IN THE PROVINCE OF BABYLON IN IRAQ

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Abstract

The purpose of the research was to determine Cryptosporidium prevalence rates in the cattle various areas of the province region of Babylon. Number of fifty fecal samples from various age groups and different sex has been collected. Conventional Process for staining fecal smears and molecular techniques using adapted Ziehl-Neelsen for detecting prevalence and assessing infection-causing Cryptosporidium species. The overall prevalence of Cryptosporidium infection by conventional approach was 16%. Nested PCR was conducted samples in which the DNA of Cryptosporidium was observed of the samples targeting the 18S rRNA gene (22%).

Article History

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1. Introduction

Cryptosporidium spp. is a compulsory intracellular (extra-cytoplasmic) protozoan parasites which is worldwide Distribution May infect a wide variety of animals and humans (Ryan et al., 2014). The cattle are believed in order to be the primary of zoonotic diseases to the human beings (Chalmers and Katzer, 2013). It is important to remember that Cryptosporidiosis induces acute or recurrent gastrointestinal disorders in cattle, which are recognized as the main of the causing watery diarrhea in abundance, loss of weight and retardation of development, decreased milk yield, morbidity and, in serious situations, morbidity, mortality. This contributes to substantial losses in economic terms (Zhang et al., 2013). The diseases is spread predominantly via the fecal to oral pathway, often through nutrition or water polluted through contact with infected animals, with oocysts, or unintentionally in laboratories (Bouzid et al., 2013). Standard approaches to infection diagnosis are based microscopic identification of oocysts of Cryptosporidium in fecal samples, however separate Cryptosporidium spp. This approaches cannot be distinguished. Centered morphometric or other phenotypic traits, owing lack of morphological distinguishing features (Stark et al., 2011). Consequently, PCR-based the techniques of molecular diagnosis can be diagnose and classify of genotypes. The molecular methods may recognize more accurate descriptions the specificity of host-adapted, propagation mechanisms with zoonosis ability of parasite (Checkley et al., 2015). Cattle with four species of the parasite are usually infected. Including Cryptosporidium bovis, Cryptosporidium parvum,
Cryptosporidium andersoni and Cryptosporidium ryanae (Abeywardena et al., 2015). It is critical that their prevalence and distribution can be estimated using molecular tools in specific geographic regions, given the diversity and zoonotic capacity of Cryptosporidium species in livestock (Yap et al., 2016). The aim of this research was explore the occurrence and consequences of Cryptosporidium infections and effected the sex, age and study area by Traditional and Molecular cattle identification methods in the province of Babylon, Iraq.

2. Materials and Methods

Fifty samples of the cattle feces from various in both sex, the age groups and from various areas of the province of Babylon were obtained from each batch, using the acid-fast stain as the primary technique for diagnostic of the oocysts. For the period from the start of June 2020 until the end of September 2020.

**Nested PCR molecular diagnosis (nPCR)**

The nested PCR process was conducted in order to diagnose of the parasite. Gene based on 18S ribosome rRNA from the fecal of bovine. It was this process conducted using the method described in Paul et al. (2009), included which fecal extraction of DNA samples using the AccuPrep @kit of extraction of fecal DNA package (Bioneer, Korea) (Table -1).

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptosporidium sp.</td>
<td>Forward AGACGGTAGGGTATTGGCCT</td>
<td>426bp</td>
</tr>
<tr>
<td>18S rRNA gene</td>
<td>Reverse TACGAATGCCCCCAACTGTC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nested Forward ATTGGAGGGCAAGTCTGGTG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nested Reverse TACGAATGCCCCCAACTGTC</td>
<td></td>
</tr>
</tbody>
</table>

**Statistical analysis**

Computer-assisted statistical analysis using SPSS, Yat's Chi-square test analyzed the variables (Petrie and Watson, 2006).

3. Results

**Prevalence rate of Cryptosporidium infection**

The standard microscopic (Acid fast stain) approach recorded a 16 % overall prevalence rate for infection with Cryptosporidium in cattle. On the other hand, the parasite was found by molecular analysis (PCR) (Table - 2). PCR was also shown to be positive for all positive microscopy specimens. The prevalence of the parasite found by both methods has been dramatically various (P≤0.05).

**Cryptosporidium spp. infection rate Molecular (Nested PCR) sex-related infection in cattle.**

The infected rate was 15 % and 26.6 % in male and female respectively. The results show the highly rate of infection in female than maleno significant difference (P≥0.05) (Table - 3).
Table - 2: The infection rate in cattle with conventional microscopic (Acid fast stain) method and NPCR methods for *Cryptosporidium* infection

<table>
<thead>
<tr>
<th>Host</th>
<th>Samples</th>
<th>Traditional</th>
<th>( Nested PCR ) test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive %</td>
<td>Positive %</td>
</tr>
<tr>
<td>Cattle</td>
<td>Total</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>16 %</td>
<td>11</td>
</tr>
</tbody>
</table>

Table - 3: Rate infection with *Cryptosporidium* by molecular (Nested PCR) techniques in cattle related to sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of Samples</th>
<th>Number of Positive</th>
<th>Infection Rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>20</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
<td>8</td>
<td>26.6</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>11</td>
<td>22</td>
</tr>
</tbody>
</table>

*Cryptosporidium* spp. infection rate by molecular (Nested PCR) in cattle related to age group

*Cryptosporidium* spp. has the highest infection rate according to age groups. 28.5 % for cattle in age groups 1 - 6 months. While the lowest was 15 % in age groups 6 - 12 months (Table - 4).

Table - 4: Total infection rate of *Cryptosporidium* infection by molecular (Nested PCR) techniques in cattle related to age groups

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Number of Samples</th>
<th>Number of Positive</th>
<th>Infection Rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6 months</td>
<td>21</td>
<td>6</td>
<td>28.5</td>
</tr>
<tr>
<td>6-12 months</td>
<td>13</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>1-3 year</td>
<td>16</td>
<td>3</td>
<td>18.7</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>11</td>
<td>22</td>
</tr>
</tbody>
</table>

No significant difference (P≥0.05)

Infection rate of *Cryptosporidium* spp. infection in by molecular (Nested PCR) cattle related to area of study

Molecular (Nested PCR) were recorded rate of infection with *Cryptosporidium* spp. at three area of Babylon province was in Al-Mohaweal (23.5 %), Al-Hashemia (23 %) and Al-Hilla (20 %) (Table -5).
Total – 5: Infection rate of Cryptosporidium infection by molecular (Nested PCR) techniques in cattle related to area

<table>
<thead>
<tr>
<th>Area</th>
<th>Number of Samples</th>
<th>Number of Positive</th>
<th>Infection Rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Hilla</td>
<td>20</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Al-Mohawal</td>
<td>17</td>
<td>4</td>
<td>23.5</td>
</tr>
<tr>
<td>Al-Hashemia</td>
<td>13</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
<td><strong>11</strong></td>
<td><strong>22</strong></td>
</tr>
</tbody>
</table>

$X^2 = 0.9615$

P value = 0.0786

No significant difference (P≥0.05)

The nPCR

Samples from genomic DNA collected from bovine fecal samples have been exposed to nested using 18S rRNA-specific primers, PCR molecular analysis to classify the parasite species. The nested PCR from all fifty samples, used in the analysis revealed a separate Agarose gel band (416 bp) indicating existence of Cryptosporidium spp. (Figure - 1).

![Image of Agarose gel electrophoresis](image)

**Figure - 1**: Illustration of agarose gel electrophoresis demonstrating product of the nPCR examination of the Cryptosporidium spp. small subunit ribosomal RNA gene. From the fecal tests of the animals (cattle). When M: Marker (2000-100bp), lane (1-12) showing some positive of the parasites samples with 416bp Product size of nPCR

4. Discussion

This present research report establishes the prevalence of Cryptosporidium spp. and its genotyping. Among the livestock in the various regions of the province of Babylon, Iraq. An adapted Ziehl-Neelson staining technique and nested PCR have been used to microscopy fecal samples collected from cattle. As microscopy is easier to do and is the only way to diagnose active infection, the PCR reported a higher prevalence of Cryptosporidium in this study. Superior PCR exposure was shown in an previous the patients with the clinical trial in of the Northern India and South Africa in the detection of Cryptosporidium infection (Uppal et al., 2014; Omoruyi et al., 2014).

The infected rate of the disease in the bovine (cattle) was 22 %. This finding was comparable to the findings of Benhouda et al. (2017) recorded infection rates of 40 % in young calves in Algeria and 35.5 %. In Sudanese diarrheal calves (Taha et al., 2017), the rate of infection, the prevalence
of infection recorded from dairy farms on the Qinghai-Tibetan Plateau region of China was higher than 17.0% from cattle checked in Poland (Rzezutka and Kaupke, 2013), and 18.6% from Ethiopian cattle (Manyazewal et al., 2016). The outbreak rate, on the other hand was smaller than 42.85% recorded in Kut city (Mohammed et al. 2016), 47.68% in Northeastern China's pre-weaned dairy calves and 52.2% in Algeria's neonatal calves (Aouatif et al., 2018). Infection ratio this may be due to gaps in management schemes, rearing methods, cattle age and race, the environmental conditions, methods of sampling and the sizes of sample, as well as screening strategies used at various research sites.

Depending on host age and geographic range, it is known that the species and genotypes of parasite infecting cattle differ (Ng et al., 2011). To provide a wider picture of Cryptosporidium infections in cattle in Iraq, our study included cattle with a wider age range (<6 months to 3 years of age) sampled from different regions of the province of Baghdad, as this study focused more on calves <1 month to 6 months of age. Cryptosporidium bovis, Cryptosporidium anderson and Cryptosporidium ryanae. Possibly, they are less pathogenic than Cryptosporidium parvum. This results in low-grade infection and decreased development of oocysts, thus reducing the burden of infection among infected animals (Helmy, 2014). Our results are in line with other research carried out in other nations (Thompson, 2016; Ng, 2017) and thus confirm, for the first time in Sudan, a similar age-dependent pattern of infection. Cryptosporidium andersoni in older calves and adult cattle, infection typically occurs (Cai et al., 2017).

5. References


