Isolation of *Clostridium perfringens* from fecal samples of captive wild animals from Baghdad Zoo Iraq and its implications

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Abstract

*Clostridium perfringens* is a Gram-positive bacteria, non-motile, spore-forming anaerobic bacterium, which is widespread in soil, feces, feed and the intestinal tract of diseased and healthy animals. The study was made to the analysis of fecal samples of Zoo animal to check the prevalence of *Clostridium perfringens*. In this study the isolated *Clostridium perfringens* from fecal samples of Zoo animals in Baghdad city. A total of 170 fecal samples was aseptically collected from different mammalian species for the isolation and identification of *Clostridium perfringens*. All the collected samples were inoculated in sheep blood agar media and incubated anaerobically in an anaerobic jar with the gas bag for the study of their cultural properties. Identification of *Clostridium perfringens* done by using Gram’s staining, cultural characteristic on blood agar and cooked meat media, motility test and biochemical tests. In this study the prevalence of *Clostridium perfringens* was 100% that all fecal samples obtained give positive results for *Clostridium perfringens* isolation.

Keywords: *Clostridium perfringens*, zoo animals, fecal samples, Baghdad zoo

1. Introduction

People went to the zoo for both entertainment and educational [1] although people become access to some animals like monkey, sheep, goat, blue peafowl, zebra foal and this contact make them at risk due to transmission of many zoonotic bacterial diseases like E.coli O157:H7, Salmonellosis, Yersinia, campylobacter, mycoplasma and clostridium spp [1-4]. *Clostridium perfringens* is a gram positive bacteria, anaerobic, commensal in the animals gastrointestinal (GI) tract without being associated with disease, as soil and feedstuffs seem to be natural habitats for these organisms [5] although this bacteria is responsible for a spectrum of diseases. *C. perfringens* enterotoxin (CPE) is thought to be an important virulence factor in animals with *C. Perfringens*-associated diarrhea [6, 8]. *Clostridium perfringens* causes several diseases in human like food poisoning which is caused by two type, type A responsible of mild food poisoning while type C causes severe food poisoning lead to necrotic enteritis [9] because of its toxins [10] while in veterinary causes enteritis, necrotic enteritis, in many species of animal especially domestic animals (horses, chicken, cow, goat, sheep) [11-16], and it is the causative agent of gas gangrene in animals [17]. In contrast, surveys of *C. perfringens* shedding by wild animals are still rare and mainly limited to a few studies in wild animals so the aim of the present investigation was to identify and characterize the *Clostridium perfringens* in fecal samples of healthy Zoo animals.

2. Material and Methods

Fecal Sample

The study was conducted at the Al-Zawraa Zoological Gardens of Baghdad city and the present study was conducted to document information on the existence of *C. perfringens* in fecal samples collected from of wild animal enclosures at the zoo. The sampled animals are listed in Table 1 and the experimental design was in such a way that samples should be collected from members of species that had five or more representatives in an enclosure. A total of 174 freshly voided fecal samples was collected aseptically with wide mouth container and transport the fecal material to zoonotic diseases laboratory in College of veterinary medicine of Baghdad University.
Isolation of *Clostridium perfringens*

1gm of fecal material diluted by adding 9 ml of sterilized normal saline then one loopful was plated on sheep blood agar and incubated at 37°C for 24 hrs under strict anaerobic conditions (Anaerobic Jar with a gas pack system). The presumptive detection of isolated bacteria was carried out by Gram staining, capsule staining and cultural characteristics of bacteria in special fluid media such as F Robinson Cooked meat medium, the confirmed test is performed by the detection of motility and gelatin liquefaction \[18, 19\].

3. Results

Isolation and identification of *Clostridium perfringens* from fecal samples

*Clostridium perfringens* isolated and identified from collected fecal samples of all 22 captive wild animals *viz* (Bear, Deer, Pony, ion, lk, og, Horse, Wildcat, Zebra, Siberia monkey, Ostrich, Baboon monkey, Kangaroo, wolf, Camel, Fox, Porcupine, Lama, Goat, jaguar, Chicken, Hyena (Table 1)

<table>
<thead>
<tr>
<th>Samples No.</th>
<th>Animal species</th>
<th>Number of fecal samples</th>
<th><em>Clostridium perfringens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bear</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Deer</td>
<td>9</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Pony</td>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Lion</td>
<td>22</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Elk</td>
<td>8</td>
<td>+</td>
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<tr>
<td>6</td>
<td>Dog</td>
<td>8</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Horse</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Wildcat</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Zebra</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Siberia monkey</td>
<td>9</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Ostrich</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Baboon monkey</td>
<td>9</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Kangaroo</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>Wolf</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>Camel</td>
<td>9</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>Fox</td>
<td>6</td>
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<tr>
<td>17</td>
<td>Porcupine</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>Lama</td>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>Goat</td>
<td>8</td>
<td>+</td>
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<tr>
<td>20</td>
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<td>7</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td>Chicken</td>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>Hyena</td>
<td>8</td>
<td>+</td>
</tr>
</tbody>
</table>

The *Clostridium perfringens* identified by its characteristic growth on blood agar it appear β-haemolytic colonies with double zone of haemolysis was observed (Fig. 1, 2).

**Fig 1:** *Clostridium perfringens* on sheep blood agar appear gray colony, flat round, β-haemolytic colonies.

**Fig 2:** *Clostridium perfringens* single isolated colony showed double zone of haemolysis.
4. Discussion

Clostridium perfringens is a part of the normal intestinal flora of humans and animals [20-23]. Although under certain conditions, C. perfringens becomes harmful and caused disease, therefore this is the first report for isolation and identified of, C. perfringens in wild zoo animal in Iraq. There are fewer studies about C. perfringens that isolated from wild animal in the world because of the difficulty in obtaining fecal samples from these species and there is only a few cases reported about enteric necrosis due to C. perfringens infection in different wild animals like avian species [32-24], elephants [33, 34]. Although other researcher isolated C. perfringens from wild animals such as wild carnivorous [35], wild bear and healthy reindeer in Norwegian [35, 36], wild deer with sudden death and intestinal haemorrhage [37], wild chimpanzees The data of the present study detect the positive C. perfringens cultured from fecal samples of all wild zoo animals in Baghdad city suggesting that C. perfringens is commonly part of the microbiota of these animals, as would be suspected also these C. perfringens needed further studies with specific primers to detect C. perfringens types and its toxins as well as these results show that prevalence of C. perfringens slightly differs from other studies which obtained 80% in wild chimpanzee [38], and 76.5% in wild carnivorous [38] this difference is because of the populations having various living conditions, being influenced by their diet and environment.

In conclusion C. perfringens was found in the feces of the healthy wild animal in high prevalence and direct culture method was good technique for isolation and identification of C. perfringens we recommended to done further studies by using PCR technique.

5. Acknowledgements

The authors wish to thank the veterinarian staff and workers of the Al-zawraa zoo for their help.

6. References

23. Wang RF, Cao WW, Cerniglia CE. PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. Applied and Environmental


