Clinicopathological changes associated with Campylobacter jejuni infection in broilers

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ABSTRACT
The present study aimed to investigate the prevalence of Campylobacter jejuni and its clinicopathological changes in broiler chickens in Sharkia province. About 50 diarrheic broiler chicks’ cloacal swabs were collected for bacteriological examination. Out of 50 examined swabs, 12 (24%) were positive for Campylobacter [C. coli 4 and 8; C. jejuni]. Isolated Campylobacter was sensitive to neomycin and gentamycin. About 45 healthy one-day-old broiler chicks received 5 mg pefloxacin/kg BW for 5 days to exclude bacterial infections. At 14th day broilers were divided into 3 groups (15/each). First group: healthy broilers non-treated (control), broilers in 2nd and 3rd groups were infected with C. jejuni. 2nd group were infected and non-treated, while 3rd group infected, and treated with 15 mg neomycin/kg BW. In drinking water for 5 days. At 1st, 3rd, and 4th day post treatment cloacal swabs were collected for re-isolation C. jejuni besides blood samples were collected for hematobiochemical study. Infected broilers showed offfood, depression, ruffled feather, diarrhea and mortality rate 40% beside significant decrease in body performance, total protein albumin and non-significant decrease in globalin coupled with non-significant elevation in RBCs, HB, PCV%, significant elevation in WBCs, ALT, ALP, urea and creatinine. Treatment infected broilers by neomycin lead to disappear clinical sign, reduced mortality rate and improved hematobiochemical parameters. It could be concluded that Campylobacter infection induces reversible adverse effect on body performance and hematobiochemical parameters. Neomycin is highly curative against Campylobacter

1. INTRODUCTION
Poultry has become an important source of meat in developing countries. Enteric disease in broilers is a common and important illness beside a risk for poultry industry in world (Kaakoush, et al. 2015). Campylobacter caused gastroenteritis is caused by two closely related species (Campylobacter jejuni and Campylobacter coli) but Campylobacter jejuni is the more predominant (Leonard, et al. 2020). Campylobacter can appear in broilers as early as 14-day age at rearing with low percentage and increase to a high percentage at the end of grows out period (Evans, 2012). Most common routes of transmission are faecal-oral ingestion of contaminated food, water and eating of raw meat. Foods implicated in campylobacteriosis (Skarp, et al. 2016). Campylobacter infection is a wide range of avian spp. and rarely transmits vertically from parents to chicks (Huang, et al. 2017). Campylobacter cause diarrhea and health problem contributing substantially to childhood morbidity and mortality (Zhang, et al. 2018). Campylobacters are small and slender gram -ve spiral shaped rods beside its food and water-borne zoonotic diseases (Anessa and Mohamed, 2019)

Antibiotics are used for bacterial infections (Thoromgsuwanakij, et al. 2018). Campylobacterosis is treated by antibiotics as aminoglycoside which act by irreversible inhibition bacterial ribosomes and impairs protein synthesis of bacteria (Fernandes and Marten, 2017). Neomycin is a member of aminoglycoside antibiotic against G +ve and G -ve organisms (Gupta and Plazomicin, 2017). The aim of the present study was isolate, identify Campylobacterand its prevalence in broilers in Sharkia province beside its effect on body performance, hematobiochemical parameters with trial of treatment was studied.

2. MATERIAL AND METHODS
2.1. Isolation and identification of Campylobacter spp
About 50 diarrheic chicks' cloacal swabs were taken from different cities of Sharkia Province. Swabs were collected aseptically and inoculated into charcoal cefoperazone desoxycholate agar medium (selective medium for isolation of Campylobacter). Plates were incubated at 37°C for 72 hrs under special microaerophilic condition (85 % nitrogen 5% oxygen, 10% carbon dioxide) (Murray, et a. 2003). Suspected colonies were identified and Bio typing by Gram staining, oxidase test, catalase test and standard biochemical methods (Atabay and Corry, 1997).

2.2. Antibiotic sensitivity test (In vitro)
Susceptibility of isolated Campylobacter species against different chemotherapeutic agents was tested by disc diffusion method (Quinn, et al. 1994).

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2.3. Antibacterial drugs

2.3.1. Pefloxacin (Peflodad 10%) solution was obtained from Dar Al Dawa Vet and Agri Industrial Co. Ltd Jordan. Each ml contains 100 mg of pefloxacin base.

2.3.2. Neomycin sulphate 20% produced from sento care Pharma comp Egypt

2.4. Experimental broilers and experimental design

About 45 apparently healthy one day-old Hubbard broilers nearly equal in live body weight (44.27-46.83gm) and received 5 mg pefloxacin/kg bw in drinking water for 5 successive days for proving that broilers are free from any bacterial infections. Broilers were fed starter ration from Kahar Company and clean drinking water ad-libium. At 14 day of age broilers were divided into three equal groups (15/each). Gp (1) healthy chicks (control), Broilers in Gp (2) were orally infected with 0.1ml saline (15/each). Gp (2) infected broilers non treated and Gp (3) infected broilers, treated with 15 mg neomycin/kg Bw. in drinking water for 5 consecutive days.

2.5. Body weight:

Chicks were individually weighed at 1st day of age and at 4th day post treatment for estimation body weight gain and feed conversion rate

2.6. Re-isolation of Campylobacter spp.: 

At 1st, 7th and 14th day posttreatment cloacal swabs were collected for Re-isolation Campylobacter jejuni

2.7. Blood samples:

At 1st, 7th and 14th day post treatment 2 blood samples were taken. First sample was taken in a tube contain EDTA for estimation of blood picture Jain (1986).


2.8. Statistical analysis was performed by using analysis of variance (ANOVA). Duncan's Multiple Range Duncan, (1955) was used to determine differences among treatments mean at significance level of 0.05. Statistics were run using SPSS program (SPSS, 2004)

3. RESULTS

Examined cloaca swabs revealed 12 (24%) were positive for Campylobacter [4 Campylobacter coli and 8 Campylobacter jejuni]. Both Campylobacter coli and Campylobacter Jujuni were negative for gram stain, positive oxidase and positive catalase and grow on 1% glycinal, meanwhile campylobacter coli not hydrolysed Hippurate but campylobacter Jujuni hydrolysed Hippurate. (Table 1 and 2). Isolated Campylobacter was sensitive to neomycin and gentamycin (Table, 3).

Campylobacter jejuni in broilers induced clinical signs (loss of appetite, depression, ruffled feather; diarrhea and 40% mortality rate at 1st and 7th day post treatment (Table 4). Campylobacterjejuni induced significant decrease (P<0.5) in body performance, total protein, albumin coupled with non-significant changes in globulin beside non-significant elevation in RBCs, HB, PCV% associated with significant increase in WBCs, AST, ALT, ALP, urea and creatinine at 1st and 7th day post treatment. Treatment infected broilers by neomycin showed disappear clinical sing, reduced mortality rate to 20%, not re-isolate Campylobacter jejuni and improved hematobiochemical parameters at 14th day post treatment (Table 4-8).

Table 1 Prevalence and type of isolated campylobacters

<table>
<thead>
<tr>
<th>Number of cloacal Swabs</th>
<th>-ve sample</th>
<th>s/w sample</th>
<th>Type of isolated campylobacters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>38</td>
<td>76</td>
</tr>
</tbody>
</table>

Table 2 biochemical identification of Campylobacter spp in broiler chickens

<table>
<thead>
<tr>
<th>Positive cloacal swabs</th>
<th>Gram stain</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Growth on 1% glycine</th>
<th>Hippurate hydrolysis</th>
<th>Campylobacter jejuni</th>
<th>Campylobacter coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-ve</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

GS. Catalase= cat. Oxidase=Ox. Growth on 1% glycine = GG. Hippurate hydrolysis= HH

Table 3 Antibiotics sensitivity of Campylobacter isolated from broilers to (n=5)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sample number</th>
<th>Sensitive</th>
<th>Moderate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin</td>
<td>10</td>
<td>8</td>
<td>80</td>
<td>2</td>
</tr>
<tr>
<td>Neomycin</td>
<td>10</td>
<td>6</td>
<td>60</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>10</td>
<td>7</td>
<td>70</td>
<td>3</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>10</td>
<td>7</td>
<td>70</td>
<td>3</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>10</td>
<td>4</td>
<td>40</td>
<td>6</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10</td>
<td>00</td>
<td>00</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 4 Mortality of healthy and diseased broilers and reisolated campylobacter

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>Total No</th>
<th>Mortality rate</th>
<th>Resolated of Campylobacter spp post treatment (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gp (1)</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gp (2)</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gp (3)</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10/00 | 00/10 | 10/10 | 00/10 | 00/10
Facciola (2018) in broilers. Variation in 66.67% infected results revealed that infected broilers with ruffled 56% were reported by Abd El dentified by Saad (2014) who identified Campylobacter coli identified as Sanitation (Leonard, et al. 2020). Campylobacter prevalence may be due to difference in was 21.5% (Mostafa, et al. 2018). The prevalence of Campylobacter in broilers in Kaliobia was 26%. Campylobacterprevalence in broilers from Sharkia Province was 29.3% (Ashraf, et al. 2018). The prevalence of Campylobacterin Assuit Province was 21.5% (Mostafa, et al. 2018) in broilers. Variation in Campylobacter prevalence may be due to difference in sanitation (Leonard, et al. 2020).

In the present study, Campylobacterisolates were identified as Campylobacter jejuni 8 (66.67%) and Campylobacter coli 4 (33.33%). Same results were reported by Saad (2014) who identified Campylobacterjejuni in rate of 60.9% in Sharkia Province. Comparable percentages of Campylobacter jejuni56% were reported by Abd El-Tawab et al. (2015) in Sharkia Province. Identified Campylobacter jejuni in rate of 66% in Egypt (Ashraf, et al. 2018). Disc diffusion test revealed isolated Campylobacter was sensitive to neomycin and gentamycin.

Our obtained results revealed that infected broilers with Campylobacterjejuni showed clinical signs (ruffled feather, depression, loss of appetite, diarrhea, reduction in body weights and mortality rate was 40%). Diseased broilers treated with neomycin showed disappearance of clinical signs and reduction in mortality rate to 20% and not re-isolate Campylobacter jejuni. Same clinical signs were observed by Khalil (2002) in broilers infected with Campylobacter jejuni. This result was consistent with Liz, et al. (2020) who stated that broilers infected with Campylobacter jejuni showed loss of appetite, depression, diarrhea, and reduction in body weights. Neomycin is a very effective drug against Campylobacter jejuni as it caused

**4. DISCUSSION**

Infected bird with Campylobacter carry a very high bacterial concentration in their gastrointestinal tract and the main sites of colonization of Campylobacter in poultry are the caeca, colon and cloaca (Facciola, et al. 2017). Campylobacter infection is characterized by inflammatory, sometimes bloody diarrhea or dysentery syndrome (cramps, fever, and pain) (Liz, et al. 2020).

In the current study, the prevalence of campylobacter was 24%. Our results are in agreement with Khalifa, et. al. (2011) who observed that the prevalence of Campylobacter in broilers in Kaliobia was 26%. Campylobacter prevalence in broilers from Sharkia Province was 29.3% (Ashraf, et al. 2018). The prevalence of Campylobacter in Assuit Province was 21.5% (Mostafa, et al. 2018) in broilers. Variation in Campylobacter prevalence may be due to difference in sanitation (Leonard, et al. 2020).

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Our results revealed that, broilers infected with Campylobacter jejuni showed non-significant change in RBCs, Hb, PCV % and significant increase in WBCs. Leukocytosis in infected broiler may be due to inflammatory response in intestinal tract (Radostitis, et.al. 2002). Similar result in blood picture was observed by Thrall (2004) stated that broilers infected with Campylobacter showed non-significant elevation in RBCs, Hb, PCV% and significant leukocytosis. Campylobacter induce significant elevation in leukocytic count in broilers (Lavini, et al. 2016).

In the present study, campylobacter infection induced significant decrease in total proteins, albumin and non-significant decrease in globulin. Reduction in total protein and albumin in broiler infected with campylobacter may be due to liver damage by campylobacter toxins in which liver is the sole site of albumin synthesis (Latimer, et al. 2003). Hyperalbuninemia in infected broilers may be due to inappetence and male absorption of nutrients from inflamed intestine (Thrall, 2004). Campylobacter induce decrease in total protein and albumin in chickens (Lavini, et al. 2016).

Our results showed that, broilers suffering from campylobacteriosis showed significant increase in AST, ALT, ALP, uric acid and creatinine. Elevation of liver enzyme, uric acid and creatinine comes from Radostitis, et.al. (2002) stated that campylobacteroxins induced degenerative changes and necrotic processes in liver and kidneys leading to increase in liver enzymes, uric acid and creatinine. These results were confirmed by result recorded by Lavini, et al. (2016) who stated that with campylobacterjejuni showed increase in liver enzymes, uric acid and creatinine in broilers.

Our study revealed that, treatment campylobacters in broilers using neomycin resulted in disappearance of clinical signs, reduction in mortality rate up to (10%), improved in body weight and not re-isolate campylobacter beside improved in hemato-biochemical parameters to normal level at 14th day post treatment. Same result were reported previously by Hassanain, (2011) in broilers infected with campylobacter and treated withbenazolin. Our results were reinforced by Agnes, et al. (2012) who observed an improvement in broilers infected with campylobacter and treated with neomycin.

5. CONCLUSIONS

It could be concluded that Campylobacter jejuni induce many changes in haemato-biochemical parameters in broilers but neomycin in therapeutic dose was effective in medication of campylobacters infection in broiler chickens.

6. REFERENCES

22. Khalifa, N.; Radwan, E and Sobhy, M (2011) molecular study of campylobacter jejuni isolated from chicken, dairy cattle and human to determine their zoonotic importance.Amer J of Res Comm. 43(3) 229-239