Evaluation of Adverse Effects of Antibiotics on Broiler Chickens

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ABSTRACT
To evaluate the impact of uncontrolled use of veterinary drugs on broilers in eastern Algeria, an experimental plan was developed for the evaluation and identification of drug toxicity in 60 chickens (30 treated and 30 non-treated with antibiotics) using analysis of serum biochemical parameters, autopsy, morpho-metric and histopathological analysis of certain internal organs. The results of the serum biochemical analysis revealed that the uric acid and aspartate aminotransferase values in antibiotic-treated chickens were high, while the lesion status showed a dominance of respiratory lesions, followed by digestive lesions, particularly hepatic lesions. The morphometric study of the internal organs (liver, kidney, and intestine) demonstrated that abnormal liver appearance was very important with minor atrophic changes in the kidney, while the histopathological examination of the liver revealed the presence of deposition in the center of the hexagons in the apical area with an apparent homogeneous structure of fibrous connective tissue. Also, there were apparent deep sinus defects in peripheral areas with an overload of fibrin. The histopathological examination of the kidneys revealed proximal tubular atrophy in the renal parenchyma along with loss of distal intratubular consistency to the peripheral zone of homogeneous structure persuading the peripheral edema. It is concluded that the uncontrolled use of antibiotics in the poultry industry leading to a moderate to severe toxicity.

Key words: Adverse effects, Antibiotics, Broiler chicken, Self-medication

INTRODUCTION
According to the Swiss Toxicological Information Centre (Curti et al., 2009), drugs are responsible for more than 20% of animal toxicity cases, with varying degrees of severity. Drug toxicity in broilers, especially due to antibiotics, is an important issue that has severe economic consequences for the poultry industry and negative impacts on human and animal health. Throughout the world, antibiotics are used in the poultry industry for preventive and therapeutic purposes. The massive use of antibiotics in poultry farming is due to their growth promotion effects and the high prevalence of self-medication (Berghiche et al., 2018a). In Algeria, poultry farmers use high doses of antibiotics to fatten chickens and save money without consulting a veterinarian. In addition, poultry farmers do not respect the withdrawal period of antibiotics, thus the poultry products are consumed by humans before the drug residues are removed from the body of the birds (Berghiche et al., 2019). The objective of the present study was to assess the impacts and risks associated with the use of antibiotics in broiler chickens.

MATERIALS AND METHODS

Ethical approval
The experiment was carried out according to the National Regulations on Animal Welfare and Institutional Animal Ethical Committee.

Animals
The experimental study was conducted on 60 broiler chickens (Ross 308) aged 7 to 8-week-old. The chickens were divided into two groups (30 treated and 30 non-treated) raised on the floor, on straw bedding in non-air-conditioned greenhouse buildings.

Treatment plan
Both groups of animals received the treatment including three types of medication administered orally: antibiotic erythromycin (Vigal 2 X, Ceva production, Algeria), anticoagulant sulfaquinoxaline sodium
(Coccidiopon, Avico production, Jordan), and a vitamin and mineral supplement (Amin‘Total, Laprovet, France). The chickens were treated according to the following plan:

Day 1 to Day 5: Vitamin supplement (1 gram per 2 liters of water) + Antibiotic (1 gram per 1 liter of water).
Day 8 and 9: Vitamin supplement (1 gram per 2 liters of water).
Day 10 to 12: Amin‘total (1 gram per 1 liter of water).
Day 15 and 16: Vitamin supplement (1 gram per 2 liters of water).
Day 17 to 19: Antibiotic (1 gram per 1 liter of water).
Day 22 to 23: Vitamin supplement (1 gram per 2 liters of water).
Day 24 to 28: Antibiotic (1 gram per 1 liter of water) + Anticoccidial (1 gram per 1 liter of water).

The chickens were fed on diets supplemented by 0.1% of antibiotic Vigal 2 X only for sick chicken and healthy chickens were fed on a basal diet without any supplementation.

Biochemical analysis
To biochemical analysis, 1 mL blood of 60 broilers was taken from the cutaneous ulnar vein using a syringe and disposable needle. The blood samples were collected in dry and heparinized tubes. The serum was separated and used for biochemical analysis. The biochemical parameters including creatinine, uric acid, alanine aminotransferase (ALAT), and aspartate aminotransferase (ASAT) were measured in a human medicine laboratory.

Morphometric, macroscopic, histological and histopathological studies
Sixty animals were euthanized and immediately dissected to maintain the integrity of the organs and tissues. The intestines carefully removed to avoid the risk of crushing and then placed in vials filled with fixative (10% formaldehyde) (Khenenou et al., 2019). The necropsy examination was carried out according to the classical technique (Khenenou et al., 2019; Berghiche et al., 2018c). The morphometric study consisted of measuring the fresh weight of the liver and kidney using a precision balance, also, the length and width of the liver and kidney using a caliper.

Statistical Analysis
The statistical analysis was performed using PAST software (Palaeontologia Electronica, Norway, Version 6.0, Free edition). The results were considered statistically significant at a significance level of p<0.5.

RESULTS AND DISCUSSION

Biochemical and biological modifications in broilers
Serum biochemical analysis revealed a significant increase (p<0.01) in uric acid and ASAT values in treated sick chickens compared to the healthy group (Table 1). Whereas the treated chickens did not show any significant change in serum creatinine and ALAT concentrations compared to the healthy group.

Macroscopic and morphometric aspects of tissues in different systems
Necropsy analysis
In the autopsy of 194 dead chickens collected from the study farm, the respiratory lesions represented a 30.15% incidence rate, followed by the digestive lesions (25.59%), particularly the hepatic lesions (14.95%). The locomotor and splenic lesions had a rate of 24.48% and 14.69%, respectively. The heart lesions were observed in nine cases (5.08 %) (Table 2).

Morphometric study
Our analysis showed very significant morpho-histological changes in the liver with minor changes in the kidney (Table 3).

Histopathological analysis
Microscopic changes of the liver
Microscopic examination of the broilers’ liver revealed lesions in the peripheral parts and lobules due to the action of antibiotics. The presence of deposition in the center of the hexagonal surface in the apical zone, an apparent homogeneous structure of fibrous connective tissue, and apparent degeneration in the peripheral area with clear edema were observed (Figures 1 and 2).

Microscopic changes of the intestine
The congestion related to stasis was demonstrated in different parts of the intestine. lesions consisted of degeneration of enterocytes, the presence of significant edema, hydropic degeneration with functional repercussions (Figure 3).
Microscopic changes of kidney

Active congestion related to hyperemia was observed that is the indicator of chronic tubulointerstitial nephritis with the toxic origin. Microscopic examination of the renal cortex demonstrated hydropic degeneration of the tubular system resulting from drug intoxication caused by self-medication. The microscopic examination of proximal convoluted tubule showed areas of degeneration with necrosis points; caryolysis and picnosis (Figure 4).

Critical points for the use of broiler chicken as an experimental model in toxicology

Broiler chicken is not a suitable experimental model to determine drug toxicity by antibiotics because the harmful effects of the antibiotics are usually chronic, while the production cycle in broilers does not exceed two months. It is noted that there is no exact time for the appearance of an injury or adverse effect, according to Haber's rules the dose is important in the expression of effects, acute toxicity of a substance includes all the specific phenomena that occur shortly after administration of a toxic substance after a single dose, chronic toxicity is less normalized and usually involves several non-fatal doses at administration (Paris et al., 2006).

Table 1. Comparison of serum biochemical parameters of chickens fed on diets supplemented with 0.1% Vigal 2 X antibiotic (treatment) with those of group fed on a basal diet (control)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>control</th>
<th>treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td></td>
<td>0.41 ± 0.03</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>Uric acid</td>
<td></td>
<td>2.45 ± 0.09</td>
<td>2.93 ± 0.11*</td>
</tr>
<tr>
<td>ALAT</td>
<td></td>
<td>19.33 ± 0.29</td>
<td>21.17 ± 0.82</td>
</tr>
<tr>
<td>ASAT</td>
<td></td>
<td>78.15 ± 4.83</td>
<td>85.33 ± 2.57*</td>
</tr>
</tbody>
</table>

*p<0.01

Table 2. The incidence of gross lesions in the different systems of autopsied chickens

<table>
<thead>
<tr>
<th>Lesions in different systems</th>
<th>Number</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic</td>
<td>29</td>
<td>14.95</td>
</tr>
<tr>
<td>Others</td>
<td>21</td>
<td>10.64</td>
</tr>
<tr>
<td>Respiratory</td>
<td>59</td>
<td>30.15</td>
</tr>
<tr>
<td>Cardiac</td>
<td>9</td>
<td>5.08</td>
</tr>
<tr>
<td>Locomotor</td>
<td>48</td>
<td>24.48</td>
</tr>
<tr>
<td>Lymphoid system (Spleen)</td>
<td>28</td>
<td>14.69</td>
</tr>
</tbody>
</table>

Table 3. Comparison between dimensions and weight of internal organs of chickens fed on diets supplemented with 0.1% Vigal 2 X antibiotic (treatment group) with those of chickens fed on a basal diet (control group).

<table>
<thead>
<tr>
<th>Organs Parameters</th>
<th>Liver</th>
<th></th>
<th>Kidney</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>treatment</td>
<td>control</td>
<td>treatment</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>59.80 ± 4.1</td>
<td>67.33 ± 0.5*</td>
<td>14.98 ± 2.33</td>
<td>12.45 ± 1.67*</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>34 ± 11.05</td>
<td>50 ± 15.67**</td>
<td>16 ± 3.66</td>
<td>16 ± 5.05</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>10 ± 0.87</td>
<td>15 ± 4.87*</td>
<td>13 ± 1.55</td>
<td>16 ± 3.33*</td>
</tr>
<tr>
<td>Height (mm)</td>
<td>72 ± 9.33</td>
<td>79 ± 6.33*</td>
<td>21 ± 2.87</td>
<td>24 ± 1.87*</td>
</tr>
</tbody>
</table>

Age of broiler: 7th-8th weeks. Data are expressed as mean ± standard deviation. (*p < 0.05; **p < 0.01).
Figure 1. Histological appearance of liver of antibiotic-treated broiler chickens. Hepatic *degeneration* (D); edema (O)

Figure 2. Microscopic lesion of the liver of broiler chickens fed on diets supplemented with 0.1% Vigal 2 X antibiotic (x100); CV: central vein; D: tissue degeneration; PT: portal triads

Figure 3. A): Microscopic aspect of the intestine of broiler chickens fed on a basal diet (normal appearance). B): Microscopic lesions of intestine of broiler chickens fed on diets supplemented with 0.1% Vigal 2 X antibiotic. (x100); V: Vessel; Cr: Crypt; C: Congestion; E: Edema
CONCLUSION

The results showed that antibiotic treatment in chickens had effects on serum biochemical parameters and demonstrated an abnormal appearance on the liver and minor atrophic changes in the kidney. Histopathological examination of the liver, kidney, and intestine revealed the presence of remarkable changes in their histological structures. The self-medication in poultry farming impacts the animal and human health and the attention of
veterinarians should be focused on the fight against self-medication in poultry farming, in particular antibiotics.

DECLARATIONS

Acknowledgments
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Competing interests
The authors have no competing interests to declare.

Authors’ contributions
Berghiche created the idea and designed the study, performed the statistical analysis, and draft the manuscript. Rahem, Labied, and Berghiche collected data. Khenenou, Boulebda, Bouzid, and Berghiche read and approved the final manuscript.

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