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Residues of cefquinome in chickens

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LIST OF ABBREVIATIONS

Abbreviation	Description
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APEC	Avian pathogenic *E. coli*
AST	Aspartate aminotransferase
AST	Antimicrobial sensitivity test
AUC	Area under the curve
B wt	Body weight
CFU	Colony forming unit
CL	Clearance
E. coli	*Escherichia coli*
EMB	Eosin Methylene Blue
FCR	Feed conversion rate
GC	Gas Chromatography
GPX	Glutathione peroxidase
H&E	Hematoxylin & Eosin
Hb	Hemoglobin concentration
HI	Haemagglutination Inhibition
HPLC	High Performance Liquid Chromatography
I/M	Intramuscular
I/V	Intravenous
IMI	Intra mammary infection
LOD	Limit of detection

LOQ	Limit of quantification
MDA	Malondialdehyde
MIC	Minimum inhibitory concentration
MRL	Maximum residue limit
MS	Gas Chromatography
ND	New castle
NO	nitric oxide
PCV	Packed cell volume
ppb	Part per billion
RBCs	Red blood cell
SE	Standard error
SOD	Super oxide dismutase
T1/2a	Elimination half life
U.A	Uric Acid
Vdss	Volume of distribution
WBCs	White blood cell

SUMMARY

The present study was conducted to evaluate the efficacy of cefquinome in treatment of broiler chickens experimentally infected with *E. coli* in addition determination of its residues using high performance liquid chromatography (HPLC) in both healthy and infected broilers.

(A) In vitro studies:

a- Isolation of *E. coli:*

Cultivation of sample of infected chickens on macConkey agar media, showed growth of pink (red) color colonies but on eosin methylene blue agar, showed green-metallic colonies after 24 hrs of incubation at 37º C.

b- Sensitivity of *E. coli* to cefquinome compared with other antibiotics (disc-diffusion method):

The results of sensitivity test showed that cefquinome had more potent inhibitory effect on *E. coli* than other antimicrobial agent tested.

(B) In vivo studies:

In our study, a total number of 200 one day old clinically healthy broiler chickens were randomly allocated into 4 equal groups (each one contained 50 chicks).

Group A: (Control group): Chicks of this group were served as non infected non treated, it administered physiological saline.

Group B: (Non Infected treated group): Chicks of this group kept non infected and treated with cefquinome (2 mg/kg b.wt, i/m), once daily for 3 successive days, intramuscularly.

Group C: (Infected non treated group): chicks of this group were experimentally infected orally with 1 ml of saline containing 10^8 CFU/ml of *E. coli* O_{78} at two weeks of age, and kept without medication.

Group D: (Infected treated group): chicks of this group were experimentally infected and treated with cefquinome.

- Blood samples (with anticoagulant, without anticoagulant) taken on 3rd, 7th and 14th day post treatment for heamatological and biochemical examination.
- Blood samples (without anticoagulant) taken on 1st, 2nd and 3rd day post treatment for immunological examination.
- Tissue samples from breast muscles, liver and kidney at 1st, 3rd, 5th and 7th day post last dost of cefquinome treatment for determination of its residues using HPLC, in addition samples from liver, kidney and intestine on 14th day post treatment for histopathological examination.

I- Effect on clinical signs, mortality rate and lesion scores:

Non infected treated group showed no clinical sings and zero% mortality rate.

Infected non treated group showed depression, off food, diarrhea, These clinical sings decreased in infected treated group.

2- Effect on serum total protein, albumin, total globulins and A/G ratio:

Cefquinome medication in healthy chicks resulted in a non significant changes in proteinogram.

Broilers infected with *E. coli* showed a significant decrease in serum total protein, albumin and A/G ratio with a significant increase in globulin when compared with the control.

Treatment of infected group with cefquinome displayed a high significant increase in serum total protein and albumin with a significant decrease in globulin.

b- Effect on kidney function tests:

Non-infected chickens treated with cefquinome showed non-significant changes in kidney functions compared with control group.

E. coli infected group evoked a significant increase in uric acid and creatinine compared with control group.

Treatment of infected chicks with cefquinome induced a significant decrease in uric acid and creatinine compared with infected non treated group.

c- Effect on lipid peroxidation and antioxidants enzymes:

Cefquinome administration to healthy chicks didn’t cause any oxidative stress represented by non significant changes in MDA, SOD and GPX levels allover the experiment when compared with control.

E. coli infection resulted in an oxidative stress represented by significant increase in MDA and decrease in SOD and GPX activates allover the experiment when compared with control group, cefquinome treatment resulted in decrease this oxidative stress.

V- Effect on some immunological parameters:

c- Effect on cellular innate immune response:

2- Effect on Nitric oxide and lysozyme activity:

Cefquinome administration cause non significant changes in NO and lysozyme levels compared with control.

Meanwhile, *E. coli* infection cause significant decrease in NO and lysozyme levels compared with control. Cefquinome treatment cause decrease in these elevated levels.

d- Effect on humeral immune response:

1-Effect on haemagglutination inhibition (HI) titer against ND:

Cefquinome administration cause no immunosuppressive effect on HI titer against ND.

Meanwhile, *E. coli* infection cause immunosuppressive effect on HI titer against ND, which improved after cefquinome treatment.

VI- Determination of cefquinome residues using HPLC:

The cefquinome calibration curve was prepared at concentrations of 25, 50, 100, 250, 500, 1000, 2500 ppb in blank sample, the calibration curve achieved by plotting peak areas of

cefquinome (obtained by HPLC) against the corresponding concentration.

The retention time of cefquinome was 13.745 mints with correlation coefficient 0.99921.

In the present study, Kidney contain the high residual level compared to liver and breast muscles, as the drug mainly excreted through kidney.

On the 1st day post cefquinome treatment, its residues still detected in all organs with high significant level in non infected group compared with the infected one.

On the 3rd day post cefquinome treatment, its residues not detected in breast muscles of infected treated broilers but still detected in non infected treated group till the 5th day post treatment.

On the 7th day post cefquinome treatment, its residues not detected in liver in both groups, and kidney in infected treated one, meanwhile still detected in kidney in non infected treated broilers.

VII- Histopathological findings:

Sever histopathological alterations in the organs of *E. coli* infected broilers, which decreased after cefquinome treatment.

Liver showed degenerative and necrotic changes in hepatic parenchyma. The latter placed by numerous inflammatory cells infiltrations. **Kidney** showed cystic dilatation of some renal tubules and and caseous necrosis of renal parenchyma.

Treatment of *E. coli* infected group with cefquinome ameliorated the drastic effect of the infection on histopathological findings, showing mild to moderate observations with nearly normal appearance of majority hepatic, renal and intestinal structures.

Pigeon Paramyxovirus 1 (PPMV-1) is a viral infection that is present worldwide causing high pigeon morbidity and mortality rates. PPMV-1 is enzootic and caused several outbreaks in domestic and wild pigeons in Egypt with substantial economic losses. It is important to investigate and assesment the pathogenicity and pathogenesis of field isolates of PPMV-1, in addition to the efficacy of available commercial vaccines.

The assessment of pathogenicity of PPMV-1 strain "PPMV-1-Egypt/Sharkia-2/2015" revealed that MDT was 86.4 hours using 9-11 days embryonated chicken eggs and this categorized the strain as a mesogenic. Intracerebral pathogenicity index (ICPI) using day-old chicks revealed 0.8. While, Intravenous pathogenicity index (IVPI) in ten 4–week old pigeons was found to be 2.88. The results of both ICPI and IVPI classified the viral strain in velogenic category.

First clinical signs appeared on day–2 post intracerebral and intravenous inoculation of PPMV-1. The mortality rate reached 10% and 100% in intracerebrally and intravenously infected birds, respectively. The clinical signs and postmortem lesions were more severe and the nervous signs were the predominant in the intravenously infected pigeons.

Also, PPMV-1 strain "PPMV-1-Egypt/Sharkia-2/2015" was used in experimental study to determine the infectivity and pathogenicity in infected pigeons via stimulating a natural route using infectious dose of 0.1ml 10^6 EID_{50} via occulonasal route. The infected pigeons revealed characteristic clinical pictures as early as 5 day post-infected (dpi), in which the nervous signs in form of paralysis, head tremors, and torticollis were the predominant and mortality rate reached to 62.5% within 12 days post challenge.

The postmortem and microscopical lesions were examined and the obvious and constant lesions were in the brain, kidneys and spleen.

The virus shedding was determined in the infected pigeons in tracheal and cloacal swabs from the 3^{rd} to 7^{th} days post-infection.

The efficacy of the commercially applied vaccines was investigated against challenge with above mentioned PPMV-1 (10^6 EID_{50}/0.1ml) in pigeons. Vaccine program I (vaccinated with 2 doses of LaSota with interval 2 weeks) and program II (vaccinated with LaSota and formalized inactivated PPMV-1) and program III (vaccinated with formalized inactivated PPMV-1).

The application of vaccine programs I, II, and III induced protection rates of 62.5%, 100% and 100% against the challenge with Velogenic PPMV-1"PPMV-1-Egypt/Sharkia-2/2015", respectively. The higher protection (100%) that was achieved by using vaccine program II and III against the PPMV-1 isolate revealed no virus detection in tracheal and cloacal swabs. But

program I that produced less protection 62.5% revealed virus shedding from trachea and cloaca.

The serological investigation of three vaccine programs aiming to protect against the challenge with Velogenic PPMV-1"PPMV-1-Egypt/Sharkia-2/2015" by using HI test showed detectable immune response from the second week (3-4 log.2), only for the pigeons (group 3) vaccinated with program III (formalized PPMV-1). The antibody titers were increased (3-3 and 4.7-5 log.2) in groups 2 and 3 that vaccinated with vaccine program II and II versus homologous and heterologous virus antigens respectively at the third week post vaccine application. However, there is no antibody detected in the sera of group one vaccinated with program I (LaSota). After challenge all challenged birds have detected antibodies.

From this study, it could be concluded that conventional virus pathogenicity tests like MDT, ICPI and IVPI may give variable results due to species variation. PPMV-1 virus is changing its tropism and pathogenicity consequently increased the risk of transmission to other susceptible avian species and human. Vaccination by using LaSota alone is not enough for prevention and control of PPMV-1 due to cross interaction between PPMV-1 and ND. Priming with live vaccine like LaSota strain and boostering with inactivated PPMV-1 vaccines for the rapid induction of cell mediated immunity since LaSota is live and the second to broaden the protective NDV and PPMV-1 viruses.