Histopathological and Hematological studies on the effect of Cephalosporin in Treatment of Nile tilapia (*Oreochromas niloticus*) infected with Aeromonas *hydrophila*

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Abstract:

This study was conducted to investigate the curative effect of cefquinome against *Aeromonas hydrophila* in Nile tilapia and the effect of hematological and histopathological findings. One hundred and thirty-five Nile tilapia fish were used and divided into 3 equal groups. 1st groups (gp) served as a negative control, 2nd gp was positive and infected with *A. hydrophila* while 3rd gp was infected group and treated with Cefquinome. The clinical signs improved in 3rd gp compared to 2nd gp and the mortality was decreased from 66.6 % in 2nd gp to 17.7 % in 3rd gp. Cefquinome treatment showed an improvement in all hematological parameters and histopathological findings of experimently infected Nile tilapia in a time dependant manner. It could be concluded that, cefquinome is effective in treatment Nile tilapia fish against *Aeromonas hydrophila* infectioin with marked improvement in the health status and histopathological as well as hematological findings.

Key words: cefquinome, hematology, histopathology, bacteria, fish.

Introduction:

Tilapia consider one of the most important aquaculture species because of their rapid growth and high tolerance. The fast expansion of tilapia farming may associate with or predispose to bacterial infectious diseases. Antimicrobials are essential role in the control of these infections ***(Shan et al., 2015)***.

Antimicrobials are important in health management of aquatic animals and improve aquatic productivity. Problems of using aqua-drugs regarding their application, withdrawal period and correct dose still in of poor understanding that could

influences a destructive hygiene and inhibit defense mechanisms ***(Miah et al., 2016).***

Antibiotics are used in the aquaculture to treat infections caused by *Aeromonas hydrophila, Aeromonas salmonicida, Edwardsiella tarda, Pastuerella piscidida, Vibrio anguillarum, Vibrio salomonicida* and *Yersinia ruckeri*. The extensive use of antibiotics in fish farming has been considered so dangerous in fish production. The major problems might be antibiotic residues and the development of antimicrobial resistant bacteria that may be transferred to consumers ***(Joan et al., 2011).***

The current study aimed to evaluate the efficiency of cefquinome in the treatment of infected Nile tilapia by *Aeromonas hydrophila* and their associated hematological and histopathological findings.

Materials and methods:

A total number of 135 Nile tilapia (*Oreochromis niloticus*) were used in this study. Fish were divided into 3 equal groups (each group contained 45 fish that subdivided equally in three glass aquaria as replicates). 1st groups (gp) served as a negative control, 2nd gp was positive and infected with *A. hydrophila* while 3rd gp was infected group and treated with Cefquinome.

Experimental infection was done by intraperitoneal injection of 24 hrs broth culture of *Aeromonas hydrophila* (0.5 ml of 1×10^7 CFU per fish) ***(Alyahya et al, 2018).***

Treatment was done by cefquinome sulfate (Cobactan® 2.5%) injectable suspension (50 ml). Every 1 ml of suspension contains 29.64 mg cefquinome sulphate (equivalent 25 mg cefquinome). (Purchased from Intervet International GmbH-Germany Company). Cefquinome had been injected in a single intramuscular therapeutic dose soon after appearance of disease symptoms, by at 3rd day of experimental infection with *A. hydrophila*. The dose of cefquinome was 10 mg/kg. b.wt. ***(Shan et al., 2015).***

The experiment was extended 2 weeks where clinical findings including mortalities were recorded throughout the experiment and sampling was done for hematological analysis and histopathological examinations.

Hematological analysis:

Blood samples were collected from caudal veins in dipotassium salt of EDTA tubes of five fish of each group at 1^{st} , 7^{th} and 14^{th} days post administration of drugs. Total erythrocytic and leukocytic counts were performed using the improved Neubauer chamber with Natt and Herrick solution as diluting fluid

according to the method described by ***Natt and Herrick (1952).*** Hemoglobin determination was done according to ***Larsen (1964).***

Histopathological examination:

Specimens from examined organs (kidneys, liver, gills, spleen, skin and muscles) of Nile tilapia were collected at 3rd 4th day and 11th day post experimental infection and 1st, 7th, and 14th day post medication, then fixed in formalin buffered solution 10 %, dehydrated in ascending graded alcohol concentrations then cleared with xylene and embedded in paraffin. Stained by hematoxylin and eosin stains (H&E) and examined microscopically ***(Bancroft and Stevens 1996).***

Statistical analysis:

The data were analyzed statistically for variance (ANOVA), with least significant difference (LSD) as described by ***Snedecor and Cochoran (1981)*** using computerized SPSS program (1999) version 17.0.

Results:

Clinical examination:

Tilapia in control group was seen in normal appearances. After third days of inoculation with *A. hydrophila*, all infected tilapia showed lack of appetite, bloated appearance and loss of some scales that became excessive at 7th day and 10th day post infection. The infected-treated group at 7th day post medication showed loss of scales**.** The mortality started after 48 hrs and reached top beak at the 7th day post infection in the infected non treated group (30, 66.6%), while the cefquinome medicated group showed lower mortality rate (8, 17.7%) where negative control group showed lowest mortality (3, 4.4%).

Effect of cefquinome on hematological parameters (Table 1):

At 1st day post medication:

Fish of infected group (2nd gp.) showed a non-significant decrease ($p<0.05$) in erythrocytic count in comparison to negative control group (1st gp.). While, 3rd gp (infected-treated group) showed a significant increase in erythrocytic count in comparison to 1st gp.

At 7th day post medication:

Fish of 2nd gp recorded a significant decrease ($p<0.05$) in erythrocytic count in comparison to 1st gp. On the other hand; 3rd gp showed a significant increase ($p<0.05$) in erythrocytic count in comparison to 2nd gp.

At 14th day post medication:

Fish of 2nd gp had a significant decrease ($p<0.05$) in erythrocytic count in comparison to 1st gp. While, 3rd gp recorded a non-significant increase in erythrocytic count in comparison to 2nd gp.

Influence on blood-hemoglobin concentration (Table 2)

At 1st day post medication:

A significant decrease ($p<0.05$) in Hb concentration in 2nd gp was observed in comparison with 1st gp. Fish of 3rd gp showed a significant decrease in Hb volume in comparison to 1st gp 1.

At 7th day post medication:

Fish of 2nd gp revealed a significant decrease ($p<0.05$) in Hb concentration in comparison to 1st gp. While 3rd gp showed a significant increase in Hb concentration in comparison to 2nd gp.

At 14th day post medication:

Fish of 2nd gp exhibited a significant decrease ($p<0.05$) in Hb volume in comparison to 1st gp. While those in 3rd gp showed a significant increase in Hb in comparison to 2nd gp.

Effects on Total leukocytes count (Table 3):

At 1st day post medication:

Fish of 2nd gp displayed a significant increase ($p<0.05$) in total leukocytes count in comparison with 1st gp. Fish of 3rd gp showed a significant increase in total leukocytes count in comparison to 1st gp.

At 7th day post medication:

Fish of 2nd gp recorded a significant increase ($p<0.05$) in total leukocytes count in comparison with normal control group. While those of 3rd gp showed a significant decrease in total leukocytes count compared to 2nd gp.

At 14th day post medication:

Fish of 2nd gp had a non-significant increase in total leukocytes count in comparison to 1st gp. On the other hand, 3rd gp recorded a non-significant decrease in total leukocytes count in comparison with infected non-treated group.

The effect of cefquinomeon on histopathological picture:

Control group (1st gp):

The histopathological picture to the internal organs of the control group revealed normal tissue architecture and cellular details with no remarkable pathological alteration**.**

Infected group (2nd gp):

At 3rd day post infection, gills showed congestion in the central venous sinus in the primary gill lamellae and focal hemorrhage in the gill arch. Some mononuclear leukocyte infiltrations were evident in the primary gill lamellae and gill arch **(Fig. 1)**. The skin and underlying musculature revealed edema with proliferation of melanomacrophage cells in the dermal tissue and focal infiltration of leukocytes in the musculature **(Fig. 2)**. The liver showed vacuolar degeneration and coagulative necrosis of hepatopancreatic cells. Parenchymal edema and hemorrhage were seen. Focal mononuclear cells infiltrations

were evident around the necrotic hepatocytes **(Fig. 3)**. The spleen revealed congestion and focal hemorrhage. Alternative atrophy and proliferation of melanomacrophage cells were evident with depletion of lymph follicles **(Fig. 4)**. The kidney revealed marked tubular nephrosis in the epithelium of the renal tubules where coagulative necrosis was evident in several tubular epithelium and vacuolar degeneration was seen in other epithelium. Marked congestion and focal hemorrhage were evident with few leukocytic infiltrations in the interstitial tissue. A remarkable depletion of hematopoietic tissue was observed (**Fig. 5)**.

At 4th days post infection, the gills revealed edema and congestion in gill arch and gill lamellae. The skin and underlying musculature revealed edema and focal coagulative (Zenker's) necrosis with few mononuclear cells infiltrations**.** The liver showed vacuolar degeneration of hepatocytes with congestion in the hepatic and pancreatic duct. Some hepatocytes exhibited coagulative necrosis. The spleen showed parenchymal edema and focal lymphoid depletion with minimal necrosis of melanomacrophages. The kidney showed mild tubular nephrosis of renal epithelium in the form of vacuolar degeneration. Focal interstitial hemorrhage was evident. Hematopoietic tissue revealed alternative hyperplasia and depletion with early proliferation of melanomacrophage cells **(Fig. 6)**.

At 11th days post infection, the histopathological examination of the internal organs of the fish of 2nd gp revealed more or less similar picture to those.

Infected and treated group (3rd gp):

At 3rd day post-infection (1st day post-medication), the experimented tilapia revealed similar finding to those reported in 2nd gp at 3rd day post infection. At 4th day post-infection (7th days post medication), the gills revealed congestion, and edema in the gill arch. Numerous leukocytic infiltration was seen at the base of the gill lamellae which exhibited congestion **(Fig. 7)**. The skin and underlying musculature showed edema and some melanomacrophage proliferation in the dermis with focal edema and hyaline degeneration in the muscle bundles **(Fig. 8)**. The liver showed wide spread vacuolar degeneration of hepatopancreas with marked edema. The spleen showed congestion and parenchymal hemorrhage. Minimal lymphoid depletion and atrophy of melanomacrophage centers were evident. The kidney displayed activation of

melanomacrophage cells and focal activation of hematopoietic tissue with focal tubular nephrosis mainly vacuolar degeneration of some renal epithelium **(Fig. 9)**.

At 11th day post infection (14th day post-medication), the gills showed congestion and melanomacrophage proliferation in the gill arch, while the gill lamellae revealed congestion and infiltration of mononuclear cells**.** The skin underlying musculature showed edema, focal hyalinization and mononuclear cell infiltrations as well as proliferation of melanomacrophages in the musculature **(Fig. 10)**. The liver showed congestion and vacuolar degeneration in the hepatopancreatic cells. The spleen revealed congestion in the splenic vessels and early proliferation of lymphocytes in the lymphoid follicles together with early activation of melanomacrophage cells **(Fig. 11)**. The kidney revealed hyperplasia of the hematopoietic tissue with early proliferation of melanomacrophage cells with mild focal tubular nephrosis **(Fig. 12)**.

Table (1): *Total erythrocytic count in normal and experimentally infected Nile tilapia fish with Aeromonas hydrophila with cefquinome treatment (M ±S. E).*

Group	Erythrocytic count 10^6/µL		
	1^{st} day post medication	**7^{th} day post medication**	**14^{th} day post medication**
1st control	0.756 ±0.17^{b}	1.23 ±0.19^{a}	**0.982 ±0.30**^{a}
2nd Infected	0.622 ±0.13^{b}	0.602 ±0.19^{b}	0.544 **±0.12**^{b}
3rd Infected treated	0.992 ±0.29^{a}	0.816 ± 0.08^{b}	0.722 **±0.15**^{ab}

The different letter in the same column means that there were significant changes at $p<0.05$.

* Cefquinome medication was done 3rd day post infection.

Table (2) *Blood hemoglobin concentration in normal and experimentally infected tilapia with Aeromonas hydrophila with cefquinome treatment.*

Groups	Hemoglobin concentration (gm/dl)		
	1st day of medication	**7th day post**	**14 day post medication**
1st Control	8.09 ±0.254[a]	8.27 ±0.301[a]	7.97 ±0.336[a]
2nd infected	6.03 ±0.139[b]	4.80 ±0.109[b]	4.20 ±0.267[b]
3rd infected-treated	6.34 ±0.140[b]	7.91 ±0.292[a]	8.27 ±0.223[a]

The different letter in the same column means that there were significant changes at $p<0.05$.
* Cefquinome medication was done 3rd day post infection.

Table (3): *Total leukocytes count in normal and experimentally infected tilapia fish with Aeromonas hydrophilla with cefquinome treatment (M ±S.E).*

Groups	Total leukocytic count (10^3/µL)		
	1st day of medication	**7th day post medication**	**14 day post medication**
1st Control	15.10 ±1.93[b]	14.94 ±1.68[b]	14.44 ±1.55[a]
2nd infected	40.17 ±8.35[a]	59.62 ±8.29[a]	62.56 ±7.99[a]
3rd infected-treated	41.94 ±3.93[a]	44.11 ±9.30[b]	19.26 ±3.06[b]

The different letter in the same column means that there were significant changes at $p<0.05$
* cefquinome medication was done at 3rd day post infection.

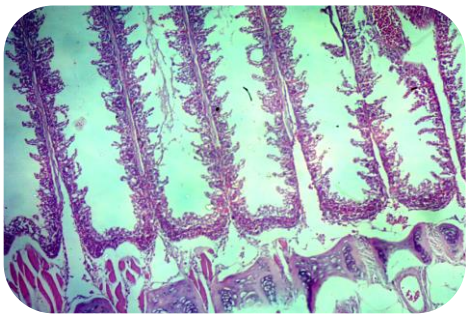

Fig (1) Group 2: at 3 days post infection, gills showing congestion in the central venous sinus and focal hemorrhage. Some mononuclear cell infilterations were evident in the primary and secondery gill lamellae. H&E stain, × 250.

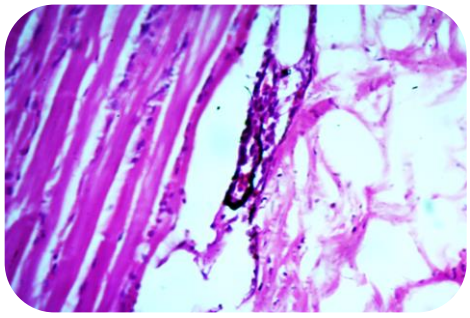

Fig (2) Group 2: at 3 days post infection, the skin and underlying musculature showing edema with proliferation of melanomacrophage cells in the dermal tissue and focal infiltration of leukocytes in the musculature .H&E stain, × 250.

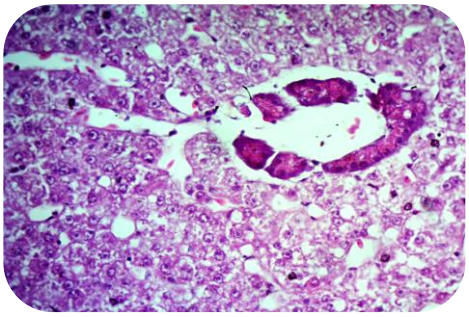

Fig (3) Group 2: at 3 days post infection, the liver showing vacuolar degeneration and coagulative necrosis of hepatopancreatic cells. Parenchymal edema and focal mononuclear cells infiltrations was evident around the necrotic hepatocytes. H&E stain, ×250.

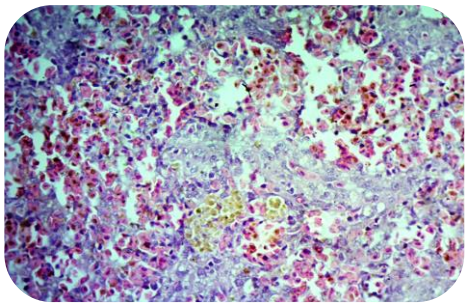

Fig (4) Group 2: at 3 days post infection, the spleen showing congestion and focal hemorrhage. with alternative atrophy and proliferation of melanomacrophage cells were evident with depletion of lymph follicles. H&E stain, × 250.

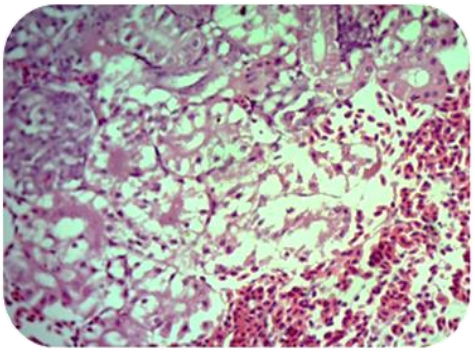

Fig (5) Group 2: at 3 days post infection; The kidney showing marked tubular nephrosis in the epithelium of the renal tubules where coagulative necrosis was evident in several tubular epithelium and vacuolar degeneration was seen in other epithelium. A remarkable depletion of hematopoietic tissue was observed. H&E stain, × 250.

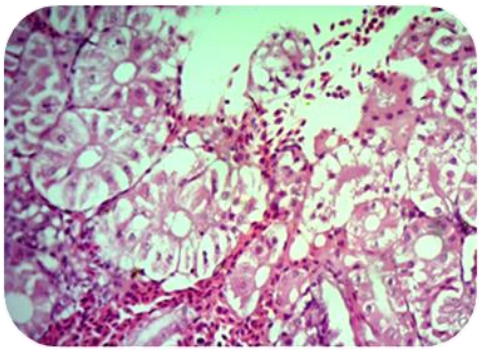

Fig (6) Group 2 at 4-day post infection; kidney showing mild tubular nephrosis of renal epithelium in the form of vacuolar degeneration. Focal interstitial hemorrhage was evident. Hematopoietic tissue revealed hyperplasia and depletion with. early proliferation melanomacrophage cells. H&E stain, × 250.

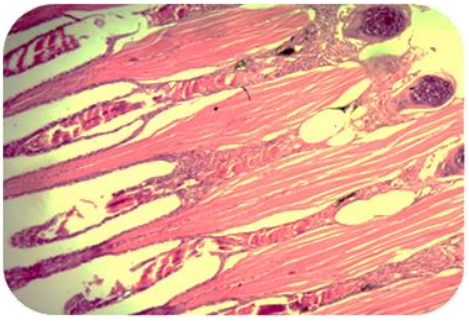

fig (7) Group3: at 4-day post infection; the gills r showing leukocytic infiltration was seen at the base of the gill lamellae which exhibited congestion. H&E stain, × 250.

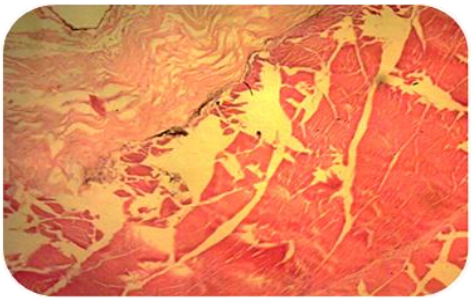

fig (8) Group3: at 4-day post infection; The skin and underlying musculature showing edema and some melanomacrophage proliferation in the dermis with focal edema and hyaline degeneration in the muscle bundles. H&E stain, × 250.

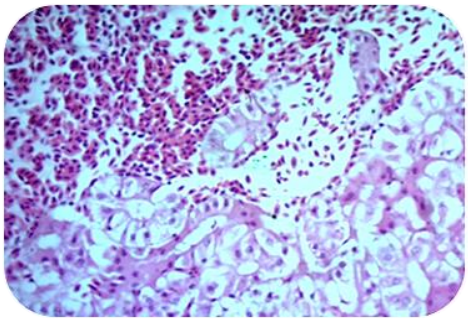

fig (9) Group3: at 4-day post infection; kidney showing activation of hematopoietic tissue with focal tubular nephrosis mainly vacuolar degeneration of some renal epithelium. H&E stain, × 250.

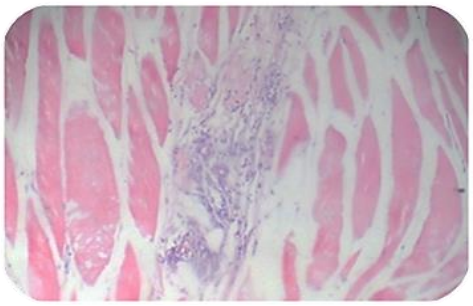

fig (10) group 3: at 11-day post infection; The skin underlying musculature showing edema, focal hyalinization and mononuclear cell infiltrations in the musculature. H&E stain, × 250.

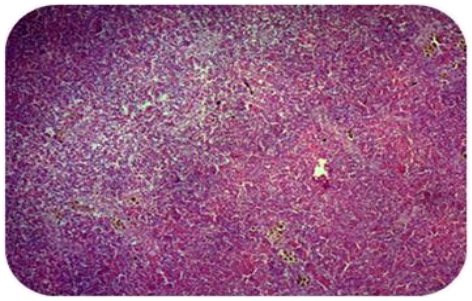

fig (11) group 3 at 11-day post infection; The spleen showing congestion in the splenic vessels and early proliferation of lymphocytes in the lymphoid follicles together with early activation of melanomacrophage cells. H&E stain, × 250.

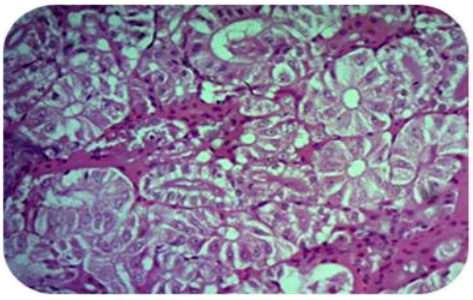

fig (12) group 3: at 11- day post infection; The kidney showing hyperplasia of the hematopoietic tissue and mild focal tubular nephrosis. H&E stain, × 250.

Discussion:

In aquaculture, antibiotics at therapeutic levels are frequently administered for short periods of time via the oral route to groups of fish; these antimicrobials must be approved by FDA ***(Romero et al., 2012).***

On the 7th and 14-day post medication, a significant

decrease in erythrocytic count and hemoglobin concentration were recorded in infected non-treated group. While the infected-treated group showed a significant increase in both erythrocytic count and hemoglobin concentration. These results resemble those obtained by ***Harikrishnan et al. (2003).*** Moreover, ***Yu et al. (2018)*** confirmed this marked decrease in the total erythrocytic count, hemoglobin concentration, hematocrit and even the erythrocyte diameter in *Aeromonas hydrophila* experimentally infected in mud loach fish. In addition to the pervious researchers, ***Abd Allah et al. (2019)*** found the same inhibitory effect of *Aeromonas hydrophila* on African catfish blood picture. ***Scott and Rogers (1981)*** explained the reason of RBCs and Hb reduction by the mobilization of the hypochromic erythrocytes from the spleen to other hematopoietic organs. ***Yardimci and Aydin (2011)*** stated that, the *Aeromonas* infection damages the internal organs (especially hematopoietic organs such as the spleen and kidney) which can also explain our hematological findings.

The leukocytic counts, on the present study, were similar to those obtained by ***Harikrishnan et al. (2003)*** in *Aeromonas hydrophila* infected fish. In a more recent study, ***Abd Allah et al. (2019)*** recorded the same inhibitory effect of *Aeromonas hydrophila* infection on leukocytic count in African catfish.

Concerning the pathological studies, ***Mu et al. (2013)*** illustrated that, the fish species when infected by *Aeromonas hydrophila* suffered from high mortality and inflammation with tissues, dropsy, red sore, necrosis, ulceration and hemorrhagic septicemia. Moreover, ***Abd Allah et al. (2019)*** recorded similar pathological lesions in *Aeromonas hydrophila* infection in African catfish; these lesions included congestion, hemolysis of erythrocytes, vacuolar degeneration a swell as coagulative necrosis of hematopoietic tissues, hepatocytes and renal tubule. More detailed histopathological picture was published by ***Yu et al. (2018)*** who investigated the histopathological changes in mud loach fish experimentally infected with *Aeromonas sobria*. Yu and his group found that the kidney exhibited extensive parenchymal hemorrhage, extensive tubular necrosis, and accumulation of proteinaceous materials in the tubular lumen and hemosiderin granules. While hepatocytes showed severely necrotic foci with karyopyknosis, karyolysis, and hyperchromatosis of the nuclear

membrane. Our histopathological findings confirm and support our investigated hematological parameters; and come in a line with the results obtained by most pervious researchers who studied the pathological consequences of *Aeromonas* infection in Nile tilapia.

Treatment with cefquinome in the present study showed a significant improvement in histopathological pictures of most tissue after 14-day post medication. These results were parallel to those obtained by many researchers as ***Abd Allah et al. (2019)*** who found similar improvement in the pathological lesions in *Aeromonas hydrophila* infection in African catfish after treatment.

The present result concluded that, cefquinome is effective in treatment Nile tilapia fish against *Aeromonas* infection through improving the histopathological and hematological findings.

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دراسات نسيجية ودموية على تأثير السيفالوسبورين في معالجة البلطي النيلي المصاب بميكروب الايروموناس

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الملخص العربى

أجريت هذه الدراسة لبحث التأثير العلاجي للسيفكوينوم ضد ميكروب الايروموناس هيدروفيللا في البلطي النيلي وتأثير ذلك على النتائج الدموية والهيستوباثولوجية. تم استخدام مائة وخمسة وثلاثين سمكة من أسماك البلطي وتقسيمها إلى 3 مجموعات متساوية. عملت المجموعات الأولى كعنصر تحكم سلبي ، وكانت المجموعة الثانية إيجابيًة حيث اصيبت بميكروب الايروموناس هيدروفيللا بينما كانت المجموعة الثالثة مصابًه بميكروب الايروموناس هيدروفيللا وعولجت باستخدام السيفينوم. تحسنت العلامات الإكلينيكية في المجموعة الثالثة مقارنة بالمجموعة الثانية وانخفض معدل الوفيات من 66,6٪ في المجموعه الثانية إلى 17,7٪ في المجموعة الثالثة. أظهر علاج السيفينوم تحسنا في جميع المعاملات الدموية والنتائج النسيجية للبلطي النيلي المصاب تجريبيا بصورة معتمده على الوقت. وعليه يمكن استنتاج أن سيفوكينوم فعال في معالجة أسماك البلطي النيلي ضد الاصابة بميكروب الايروموناس هيدروفيللا مع تحسن ملحوظ في الحالة الصحية والنتائج النسيجية وكذلك صورة الدم.