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THE CONGRUENCE OF VIRULENCE GENES AND PATHOGENICITY OF *Edwardsiella Tarda* STRAINS OF *Oreochromis Niloticus* AND *Clarias Gariepinus* ORIGIN ON *Oreochromis Niloticus*

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Abstract

Edwardsiella tarda infection is considered a dangerous septicaemic disease with high economic losses. The present work was planned to investigate the LD_{50} and pathogenicity of *E. tarda* strains isolated from two different fish species (*O. niloticus* and *C. gariepinus*) in correlation with the number of virulence genes in each strain. The results revealed that the most virulent strain was strain 1 (group 1) causing 50% mortality within 12h at concentration of 1.8 X 10^5 CFU. The clinical signs of experimentally infected *O. niloticus* with *E.tarda* of the four fish groups were recorded. The signs were clearly prominent in the fish group one infected with *E.tarda* strain containing the four virulence genes. However, the signs were also present in the other three groups containing three, two and one virulence genes respectively, with variant mild degrees, while as the control group showed no clinical changes. Some hematological and serum biochemical parameters were studied on the blood and sera of experimentally infected fish, which revealed a decrease in Hb, RBCs, PCV, total proteins, albumin and globulins and an increase in TLC, Lysozym concentration. Histopathological studies on the tissues of fish were also recorded.

Key words: *Edwardsiella tarda* virulence genes.

INTRODUCTION

The aquaculture industry grows rapidly to meet the needs of expanding global human populations. Under certain aquaculture management circumstances the incidence of the bacterial disease outbreaks, may hinder the fish industry, causing huge mortalities and in turn economic losses. *E.tarda*; the causative agent of *Edwardsiellosis*, a serious systematic disease of cultured fish,

has been recognized as one of the major pathogens that cause severe losses to aquaculture industries worldwide (Hou *et al*., 2009 and Xu et al., 2014) with a broad geographical distribution and expanded host range (Park *et al*., 2012) including humans (Xie *et al*., 2014).

Edwardsiellosis has a major economic impact on aquaculture industry under Egyptian conditions, causing massive mortalities in various fish species. *O. niloticus* isolated *E. tarda* in the summer season only while as, *C. gariepinus* allover the seasons of the year which might explain its role as a reservoir for *E.tarda* pathogen allover the year (Abd El-Kader, 2016).

The virulence profile may be a useful tool for prediction of the pathogenicity (Yamada and Wakabayashi, 1999) and used as a helpful indicators for the clinical assessment and prediction of *Edwardsiellosis* risk in fish culture environments (Li *et al*., 2011).The expression of several virulent genes (hlyA, citC, fimA, gadB, katB and mukF) correlated with the mortality of *E. tarda*-infected fish, (Shen and Chen, 2005). The hemolysin in *E. tarda* is essential for fish invasion in vivo and in vitro (Wang *et al*., 2010).

Therefore, the present work was planned to investigate the LD_{50} and pathogenicity of *E.tarda* strains isolated from two different fish species (*O. niloticus* and *C. gariepinus*) in correlation with the number of virulence genes in each strain and recording the alterations in some hematological and blood serum biochemical parameters on the blood of the experimentally infected *O. niloticus* and the Histopathological changes of the tissues of the experimentally infected fish.

MATERIALS AND METHODS

Experimental fish:

A total number of 300 apparently healthy *O. niloticus*, weighing (60± 5 g) were kept in glass aquaria, supplied with chlorine free tap water with continuous aeration according to (Innes, 1966), at the laboratory of Sakha Aquaculture Research Unit, Central Laboratory of Aquaculture Research, about two weeks for acclimation, prior to the lethal dose fifty and pathogenicity

experiments, (Plumb and Browser, 1982). Random samples were taken for bacteriological examination to ensure that the fish were *E.tarda* pathogen free. The fish were fed on a commercial fish diet containing 25% crude protein at 3% of biomass according to (Eurell *et al*., 1978) and offered twice daily.

E. tarda strains:

On the basis of the PCR and the virulence genes results; *E.tarda* strains were divided into four genotypes were taken for the LD_{50} experiments; positive for the four virulence genes strain1 isolated from *C. gariepinus* (hyl A+ gad + fim + gyr +), positive for three virulence genes strain2 from *O. niloticus* (hyl A+ gad - fim + gyr +), positive for two virulence genes strain3 from *C. gariepinus* (hyl A- gad - fim + gyr +), positive for one virulence gene strain 4 from *C. gariepinus* (hyl A+ gad - fim - gyr -) provided by (Abd El-Kader, 2016).

Evaluation of the pathogenicity of isolated _E. tarda_ strains:

Total bacterial count:

Using the pour Plate method for estimation of the number of *E. tarda* strains per 1 ml, that will be used in demonstration of the inoculum dose for experimental studies, according to (Cruickshank *et al*., 1975).

Determination of median lethal dose (LD_{50}) of _E. tarda_ strains in _O. niloticus:_

A total number of 200 apparently healthy *O.niloticus* weighing (60±5), were divided into four major groups for the four strains, fifty fish per each. Each group was subdivided into five subgroups, ten fish per each, from each subgroup the fifth group was kept as control. 24 hours colony cultures of each *E.tarda* strain on TSA were used; the colonies were picked up and suspended in sterile saline in a tenfold serial dilution and subsequent incubation at 28 ° C for 24 h for plate counts on tryptic soy agar (TSA), only the dilutions (10^3-10^6 cfu) were used. Each fish subgroup was intraperitonially injected with 0.5 ml of each bacterial dilution and kept 7 days post-inoculation for observations, mortalities were recorded daily according to (Ibrahem *et al*., 2011), re-isolation and

biochemical identification of the pathogen were carried out and the freshly dead fish were moved for P.M examination. The LD_{50} of each *E.tarda* strain was calculated according to (Reed and Muenchen, 1938).

Experimental infections:

A total number of 100 apparently healthy *O.niloticus*, weighing (60±5) were divided into five major groups 20 fish per each. For groups (1-4); 20 fish for I/P injection with 0.2ml LD_{50} of each strain respectively, the group number 5 was kept as control, 20 fish for I/P injection with 0.2ml PBS according to (Ibrahem *et al*., 2011). The observation period was 30 days post injection; blood and sera were taken for the hematological and the serum blood biochemical investigations at 0, 10^{th}, 20^{th} and 30^{th} days. Also, mortalities were recorded daily. Re-isolation and biochemical identification of the pathogen were carried out and the freshly dead fish were moved for P.M examination and histopathological studies. Fish surviving the experimental infection were sacrificed at the 30^{th} day post injection and moved for P.M examination and histopathological studies.

Histopathological examinations:

Specimens from kidney, liver and spleen of experimentally infected fish were taken. Specimens were fixed immediately in 10% neutral buffered formalin, then dehydrated in ascending grades of ethanol, embedded in paraffin wax, sectioned at 4-5 micron thickness and stained with haematoxlin and eosin (H&E), and examined by light microscope according to (Bancroft and Gamble, 2007).

Hematological investigations:

The erythrocytic and leukocytic counts, the Hemoglobin concentration determined by the cyanomet-hemoglobin method with Drabkin's solution as well as, the differential leukocytic count (DLC) among one hundred cells of blood smear, were determined according to the method described by (Stoskopf, 1993). The absolute DLC was calculated according to (Thrall, 2004), and the packed cell volume was determined according to (Dacie and Lewis, 1991).

Blood serum biochemical analysis:

Serum total proteins were determined according to (Doymas *et al*., 1981) at the wave length 540 nm, Serum albumin were estimated calorimetrically at wave length 550 nm according to (Dumas and Biggs, 1972). Globulins content were calculated mathematically, Albumin/Globulin (A/G) ratio was calculated from data of albumin and globulin concentration. Activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically at the wave length 540 nm, according to (Reitman and Frankel, 1957).

Lysozyme concentration assays:

The lysozyme activity of sera of the blood of experimentally infected fish, were assayed according to the method described by (Demers and Bayne, 1997), based on the ability of lysozyme to lyses the Gram positive lysozyme sensitive bacterium; Micrococcus lysodeikticus.

Statistical analysis:

All assays were performed in triplicate. The data were statistically analyzed using one- way or two- way analysis of variance ANOVA model of SAS soft ware version 6.12 (SAS, 1996). Duncan multiple range test was used to test the significance among the means (Snedecor and Cochrang, 1989).

RESULTS AND DISCUSSION

The lethal dose fifty (LD_{50}) experiments revealed that the most virulent strain was strain 1 (group 1) causing 50% mortality within 12h at concentration of 1.8 X 10^5 CFU, the strain 2 (group 2) causing 50 % mortality after 16 h at a concentration of 1.7 X 10^5 CFU, while the strain 3 (group 3) causing 50% mortality after 18 h at the concentration 1.4X10^5 CFU and the strain 4 (group 4) causing 50% mortality after 24h at the concentration 1.2 X10^5 CFU. Ibrahem *et al*. (2011) obtained the LD_{50} at the concentration (10^4cfu) as well as, Shabaan, (2007) recorded LD_{50} at $10^{4.5}$cfu. The differences in the LD_{50} values, between many different authors may be attributed to the number of the virulence genes in

E.tarda strains, the pathogen properties as well as size and weight of fish species and environmental conditions such as temperatures difference (Amandi, *et al*., 1982 and Ibrahem *et al*., 2011). The dead fish showed mild haemorrhages on the body, loss of pigmentation, (Fig.1). Internally, congested liver with distended gall bladder, dark congested kidney and spleen less sever distended haemorrhagic intestine, presence of yellowish ascitic fluid.

Fig. 1. *O. niloticus* injected with *E.tarda* strains in LD_{50} experiments showed mild haemorrhages on the body, loss of pigmentation, group 1= 1.8 X 10^5 CFU, group 2=1.7 X 10^5 CFU, group 3=1.4 X 10^5 CFU and group 4=1.2X 10^5 CFU.

The clinical signs of experimentally infected *O. niloticus* with *E.tarda* of the four fish groups showed, lethargy, abnormal swimming behavior including spiral movements, loss of swim axis, loss of the escape and defense reflexes, floating near the water surface with gasping (Fig. 2), and death occurs with opened mouth and flared opercula which may be due to the stress response, and development of anemia leading to insufficient oxygen in the tissues and therefore a Compensatory increased respiration (Walters and Plumb, 1980 and Mohanty and Sahoo, 2007). These findings were supported by (Meyer and Bullock, 1973; Miyazaki and Kaige, 1985 and Vasquez-Piñeros *et al*., 2010).

The gross external signs were clearly prominent in the fish group one infected with *E.tarda* strain containing four virulence genes, showing excessive mucus, scale detachment, sever haemorrhagic patches on the skin allover the body surface. Cutaneous ulcers extended allover the body especially at the dorso-lateral surface of the body and the caudal peduncle. Tail erosion and

congestion of fins, abdominal ascitis, protruded haemorrhagic anus and bilateral exophthalmia, (Fig.3-10).

However, the aforementioned external signs were also present in the other three groups containing three, two and one virulence genes respectively, with variant mild degrees, while as the control group showed no clinical changes; the successful production of the disease signs in the experimentally infected *O. niloticus* leaved no doubts about the pathogenicity of *E. tarda* toward the cultured *O. niloticus* which indeed, can get infected also by *E. tarda* isolates of *C. gariepinus*.

Internally; congestion and haemorrhagic enteritis and adhesion between organs. yellowish fibrinous ascitic fluids. Pale liver and distended gall bladder. Generalized hyperemia, swelling, congesion of the liver, kidney, spleen and gastrointestinal tract were present in all the four groups (Fig. 11 and 12).

Mortality rates of the four groups and the control group were; 90%, 80%, 75%, 65% and 0% for the groups 1, 2, 3, 4 and the control group respectively.

The recorded mortality rates of the four fish groups when compared with the control one, revealed highest mortality rate for groups; one and two which infected with *E. tarda* strains containing four and three virulence genes respectively, may be attributed to the higher number of virulence genes as well as, the synergistic effects conferred by combination of several virulence genes. (Shen and Chen, 2005) observed the expression of several virulent genes such as hlyA, fimA and gadB was correlated with the mortality of *E.tarda* infected fish.

Fig. 2. *O. niloticus* experimentally infected with *E. tarda* showed abnormal swimming behavior including spiral movements

Fig. 3. group (1) of *O. niloticus* experimentally infected with *E.tarda* strain containing four virulence genes; showed bilateral exophthalmia.

Fig. 4. group (1) of *O. niloticus* experimentally infected with *E.tarda* strain containing four virulence genes; showed excessive mucus, scale detachment, sever cutaneous ulcers extended allover the body specially at the dorso-lateral surface of the body.

Fig. 5. group (1) of *O. niloticus* experimentally infected with *E.tarda* strain containing four virulence genes; showed scale detachment, sever haemorrhagic patches on the skin allover the body surface. congestion of fins. Ascitis.

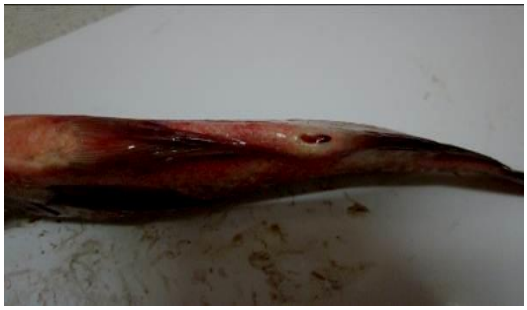

Fig. 6. group (1) of *O. niloticus* experimentally infected with *E.tarda* strain containing four virulence genes ; showed sever haemorrhagic patches on the skin allover the body surface. congestion of fins congestion ,reddening and bleeding of the anal opening .

Fig. 7. group (4) of *O. niloticus* experimentally infected with *E.tarda* strain containing one virulence gene; showed mild haemorrhagic patches on the skin allover the body surface.

Fig. 8. group (2) of *O. niloticus* experimentally infected with *E.tarda* strain containing three virulence genes; showed sever haemorrhagic patches on the skin allover the body surface, head and operculum and congestion of fins

Fig. 9. group (2) of *O. niloticus* experimentally infected with *E.tarda* strain containing three virulence genes; showed scale detachment cutaneous ulcers and unilateral exophthalmia

Fig. 10. group (3) of *O. niloticus* experimentally infected with *E. tarda* strain containing two virulence genes; showed haemorrhagic patches on the skin allover the body surface, scale detachment and congestion of fins .

Fig. 11. *O. niloticus* experimentally infected with *E.tarda* strains (group 2, 3 and 4 respectively); showed congested liver and dark congested enlarged spleen.

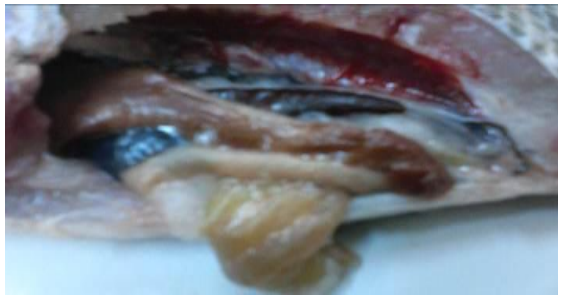

Fig. 12. group (1) of *O. niloticus* experimentally infected with *E. tarda* strain containing four virulence genes; showed congested liver with distended gall bladder , dark congested enlarged spleen and coagulated fibrinous yellowish ascitic fluids.

The RBCs counts and haemoglobin levels were decreased (Table 1). (Benli and Yildiz, 2004; Shaaban, 2007 and Vasquez-Piñeros *et al*., 2010).), probably due to *E. tarda* has developed abilities to utilize hemin, haemoglobin and hematin as iron source as well as siderophore-mediated iron uptake mechanism encode a coproporphyrinogen ш oxidase, heme iron utilization protein, hemin receptor, use hemin related iron sources (Abbott and Janda, 2006), also, genes for ferric uptake regulator, ferrous iron utilization were recorded in *E. tarda* genome (Wang *et al*., 2009).

In extra- intestinal infections with *E. tarda*, cell-associated hemolysin is also suggested as a virulence factor, in which a possible function of this hemolysin could be the acquisition of the host iron through lysis of the erythrocytes to release hemoglobin stores (Janda and Abbott, 1993).

On iron deficient medium; *E. tarda* produced siderophores which permit the pathogen to scavenge for iron in the blood of the host (park *et al*., 1983; Kokubo *et al*., 1990 and Ouyang *et al*., 1998). The ability of bacteria to acquire iron acquisition using the bacterial iron chelator, siderophore is essential for the survival and replication of the bacteria (Srinivasa Rao *et al*., 2003a,b and Igarashi *et al*., 2002). *Edwardsiella* produced products causing lysis and destruction of RBCs and reduces its number and its Hb content (Shaaban, 2007).

The reduction in hematocrit was observed in different experimental bacterial infections in fish, such as Nile tilapia infected with *E. tarda* (Benli and Yildiz, 2004 and Shaaban, 2007); due to a severe disruption of hematopoiesis by bacteria or their products. Microcytic anemia by reducing the content of hemoglobin, mean corpuscular hemoglobin and complete blood count was also reported in *O. niloticus* infected with *E. tarda* (Vasquez-Piñeros *et al*., 2010).

Table 1. The Erythrocytes, haemoglobin and packed cell volume of the blood of *O. niloticus* post infection with the four *E. tarda* strains from zero day to 30th day (end of experiment) within the same group.

Day	RBCs (x 10^6/µl)					Hb (g/dl)					PCV (%)				
	1	2	3	4	Control	1	2	3	4	Control	1	2	3	4	Control
zero	1.90^{a} ±0.5	1.91^{a} ±0.58	1.88^{a} ±0.29	1.89^{a} ±0.61	1.90^{a} ±0.2	5.88^{a} ±1.02	5.89^{a} ±0.6	5.90^{a} ±0.4	5.89^{a} ±0.25	5.90^{a} ±0.5	14.18^{a} ±0.9	14.20^{a} ±0.85	14.23^{a} ±0.5	14.17^{a} ±0.23	14.20^{a} ±0.7
10th	1.42^{b} ±0.8	1.49^{b} ±0.24	1.51^{b} ±0.27	1.58^{b} ±0.54	1.88^{a} ±0.3	5.40^{b} ±0.70	5.49^{b} ±1.04	5.49^{b} ±0.81	5.56^{b} ±1.07	5.91^{a} ±0.52	12.46^{b} ±1.02	12.54^{b} ±1.05	12.67^{b} ±0.98	12.85^{b} ±1.09	14.05^{a} ±1.01
20th	1.24^{b} ±0.4	1.30^{bc} ±0.33	1.37^{bc} ±0.6	1.43^{bc} ±0.31	1.92^{a} ±0.5	4.91^{c} ±0.9	4.91^{cd} ±0.45	4.98^{cd} ±1.07	5.03^{c} ±0.57	5.90^{a} ±0.42	11.24^{c} ±1.04	11.25^{c} ±0.9	11.28^{cd} ±0.73	11.90^{bc} ±1.22	13.99^{a} ±0.5
30th	1.10^{c} ±0.5	1.21^{c} ±0.5	1.22^{c} ±0.71	1.31^{c} ±0.02	1.90^{a} ±0.5	4.10^{d} ±0.5	4.30^{d} ±0.64	4.41^{d} ±0.8	4.49^{d} ±0.68	5.93^{a} ±0.35	10.40^{c} ±0.5	10.61^{c} ±0.83	10.69^{d} ±1.04	10.81^{c} ±1.04	14.14^{a} ±0.74

Mean ± SD Means in a column with different superscripts different significantly at $P < 0.05$.

The total leukocytic of the four experimentally infected groups of *O.niloticus* were increased when compared with the control group, this also was reported by (Benli and Yildiz, 2004) however, the opposite was reported by (Shaaban, 2007).

The increased leukocytic counts (TLC) during infection correlating organism defenses against the pathogen (Balfry *et al*., 1994 and Benli and Yildiz, 2004). The innate immunity was the principal immune system for eliminating the majority of *E. tarda* in carp (Yamasaki *et al*., 2013).The declined level of TLC of the 1st and 2nd experimentally infected groups of *O.niloticus* in the day 30th but still higher than that of the control one probably, due to the earlier lymphoid depletion caused by the higher number of the virulence genes of *E. tarda*.

The neutrophil and monocyte counts of the the four experimentally infected groups of *O.niloticus* were increased when compared with the control group; matching that reported by (Shaaban, 2007 and Vasquez-Piñeros *et al*., 2010). In bacterial infection, the predominant cell line blood is the neutrophil, with neutrophilia (Vasquez-Piñeros *et al*., 2010) and there were activation of the cellular innate immunity; increasing the macrophages and monocytes.

In experimentally infected groups of *O. niloticus*, the lymphocytic counts showed a significant increase till the day 20th, which may be attributed to the high activity of the cellular innate immunity in the first stages of infections (Yamasaki *et al*., 2013), then abruptly declined at the end of the experiments day 30th, such lymphopenia was also recorded by (Shaaban, 2007 and Vasquez-Piñeros *et al*., 2010) in experimentally infected *O. niloticus* with *E. tarda*.

Table 2. The total leukocytic count of the blood of *O. niloticus* post infection with the four *E. tarda* strains from zero day to 30th day (end of experiment) within the same group.

day	1	2	3	4	Control
zero	47.05 ^{c} ± 0.98	47.21 ^{b} ±1.02	47.34 ^{b} ± 2.10	46.77 ^{c} ± 2.03	47.31 ^{a} ±0.82
10th	55.41 ^{ab} ± 1.20	50.10 ^{b} ± 4.01	50.07 ^{b} ± 1.60	51.37 ^{b} ± 1.58	47.15 ^{a} ± 1.9 ^{a}
20th	60.60 ^{a} ± 3.01	58.56 ^{a} ±0.97	55.67 ^{a} ± 2.01	55.00 ^{ab} ± 0.89	46.97 ^{a} ± 1.88
30th	50.44 ^{bc} ± 0.86	53.87 ^{ab} ±2.30	57.84 ^{a} ±0.97	57.70 ^{a} ± 2.07	47.33 ^{a} ± 0.97

Mean ± SD Means in a column with different superscripts different significantly at $P < 0.05$.

The Lysozyme concentrations were increased in the four experimentally infected *O.niloticus* groups, when compared with the control group, which also were reported by (Caruso *et al*., 2002; Mohanty and Sahoo, 2010 and Nakhro *et al*., 2014). A possible sound explanation; The Lysozyme was mainly produced by macrophages (Gordon *et al*.,1974), indeed, the continuous activation of lysozyme gene during maturation of macrophages (Cross *et al*.,

1988), therefore, the increased TLC, phagocytes and macrophages increased serum lysozyme concentrations.

Table 3. The absolute differential leukocytic count of the blood of *O. niloticus* post infection with the four *E. tarda* strains from zero day to 30th day (end of experiment) within the same group.

Day	Heterophil (x 10^3 /µl)					Lymphocyte (x 10^3 /µl)					Monocyte (x 10^3 /µl)				
	1	2	3	4	Control	1	2	3	4	Control	1	2	3	4	Control
Zero	6.45 [b] ±0.04	6.71 [b] ±0.5	6.55 [b] ±0.09	6.69 [b] ± 0.31	6.68 [a] ±0.4	29.21 [c] ±2.01	30.4 [c] ±1.2	28.67 [c] ±0.89	30.02 [c] ±1.04	30.01 [a] ±1.3	3.2 [c] ±0.2	2.99 [c] ±0.02	3.02 [c] ±0.05	3.4 [b] ±0.4	3.1 [a] ±0.31
10th	12.5 [a b] ±0.4	11.9 [a b] ±1.02	11.5 [a] ±0.61	11.1 [a] ±0.78	6.66 [a] ±0.5	39.4 [b] ±1.8	37.09 [b] ±3.01	37.14 [b] ±0.85	36.91 [b] ±0.89	29.5 [a] ± 0.91	4.4 [b] ±0.3	3.77 [b] ±0.5	3.64 [b] ±0.3	3.59 [b] ±0.92	3.21 [a] ±0.78
20th	12.9 [a] ±0.1	12.2 [a b] ±0.4	11.9 [a] ±0.23	11.1 [a] ±0.87	6.60 [a] ±0.9	45.5 [a] ±0.65	40.23 [a] ±0.91	41.43 [a] ±2.01	40.12 [a] ±1.3	30.14 [a] ± 1.2	6.01 [a] ±0.09	4.35 [a] ±0.3	4.12 [a] ±0.25	4.06 [a] ±0.78	3.17 [a] ±0.66
30th	13.1 [a] ±0.6	12.87 [a] ±0.9	12.1 [a] ±0.78	11.5 [a] ±1.01	6.70 [a] ±1.2	21.01 [b] ±3.2	22.41 [b] ±0.21	24.6 [a] ±1.03	24.8 [a] ±3.1	30.29 [a] ± 0.99	6.1 [a] ±1.02	4.65 [a] ±0.99	4.46 [a] ±0.66	4.20 [a] ±0.22	3.11 [a] ±0.04

Mean ± SD Means in a column with different superscripts different significantly at $P < 0.05$

Table 4. The lysozyme concentrations (µg/ml) of the serum of *O. niloticus* post infection with the four *E. tarda* strains from zero day to 30th day (end of experiment) within the same group.

Day	1	2	3	4	Control
Zero	9.30 [c] ±0.78	9.31 [d] ±1.01	9.10 [d] ±0.56	9.25 [a] ±0.9	9.33 [a] ±0.14
10th	13.21 [b] ±1.3	12.87 [c] ±2.05	12.86 [c] ±0.95	14.31 [b] ±1.01	9.20 [a] ±1.03
20th	15.41 [a] ±0.95	14.51 [b] ±2.4	14.71 [b] ±3.01	16.43 [a] ±0.7	9.36 [a] ±0.8
30th	15.97 [a] ±0.5	15.78 [a] ±0.36	15.74 [a] ±0.86	17.01 [a] ±1.21	9.38 [a] ±0.61

Mean ± SD Means in a column with different superscripts different significantly at $P < 0.05$

Table 5. The total protein, albumin, globulins, ALT and AST of the serum of *O. niloticus*. post infection with the four *E.tarda* strains from zero day to 30th day (end of experiment) within the same group.

Day of sampling	Parameter	1	2	3	4	Control
Zero	Total protein	5.6± 0.12 [a]	5.40± 0.9 [a]	5.43± 0.82 [a]	5.57± 0.24 [a]	5.51± 0.7 [a]
	Albumin	2.18±0.11 [a]	2.15± 0.12 [a]	2.16± 0.14 [a]	2.16± 0.09 [a]	2.17± 0.10 [a]
	Globulin	3.42± 0.17 [a]	3.25± 0.11 [a]	3.27± 0.09 [a]	3.41± 0.08 [a]	3.34± 0.09 [a]
	Alb./Glob. ratio	0.63± 0.01 [a]	0.66± 0.01 [a]	0.66± 0.02 [a]	0.63± 0.01 [a]	0.65±0.03 [a]
	ALT	37.01± 0.5 [a]	36.71± 0.6 [a]	37.10± 0.41 [a]	37.10± 0.8 [a]	37.12± 1.01 [a]
	AST	30.4± 1.1 [a]	30.57± 1.02 [a]	29.23± 0.09 [a]	29.88±1.04 [a]	30.07± 1.02 [a]
10th	Total protein	5.00± 1.5 [b]	5.01± 1.24 [b]	5.29± 1.6 [ab]	5.31± 0.99 [ab]	5.54± 1.23 [a]
	Albumin	2.09± 0.09 [b]	2.10± 0.09 [b]	2.14± 1.01 [ab]	2.23± 0.5 [a]	2.18± 0.7 [ab]
	Globulin	2.91± 0.14 [c]	2.85± 0.12 [c]	3.10± 0.1 [b]	3.41± 0.5 [a]	3.42± 0.11 [a]
	Alb./Glob. ratio	0.74± 0.01 [a]	0.70± 0.02 [ab]	0.65± 0.02 [b]	0.69± 0.01 [ab]	0.65± 0.03 [b]
	ALT	37.03± 2.3 [a]	37.00± 3.01 [a]	37.25± 2.11 [a]	37.09± 2.2 [a]	37.09± 1.97 [a]
	AST	30.46± 1.09 [ab]	30.59±2.01 [ab]	31.10± 0.95 [a]	29.89± 1.9 [b]	30.41±1.8 [ab]
20th	Total protein	4.8± 1.03 [c]	4.91± 2.1 [b]	4.87± 0.98 [b]	4.90± 0.25 [b]	5.50± 0.5 [a]
	Albumin	1.89± 0.9 [bc]	1.99± 0.5 [c]	1.86± 0.7 [bc]	2.01± 0.24 [b]	2.20± 0.65 [a]
	Globulin	2.91± 0.1 [ab]	2.84± 0.14 [b]	2.89±0.12 [ab]	3.00± 0.11 [ab]	3.41± 0.1 [a]
	Alb./Glob. ratio	0.59± 0.05 [b]	0.64± 0.04 [ab]	0.60± 0.02 [b]	0.65± 0.04 [ab]	0.67± 0.07 [a]
	ALT	39.80± 3.1 [a]	40.01± 2.01 [a]	39.83± 1.98 [a]	39.01±1.01 [b]	37.12± 2.01 [c]
	AST	36.41± 1.1 [a]	35.31± 0.99 [ab]	35.04± 0.97 [b]	33.07± 1.02 [c]	30.11± 1.04 [d]
30th	Total protein	4.4± 0.9 [c]	4.7± 0.7 [b]	4.71± 0.51 [b]	4.79± 0.22 [b]	5.57± 0.54 [a]
	Albumin	1.8±0.1 [c]	1.86± 0.09 [b]	1.89± 0.11 [b]	1.97± 0.5 [b]	2.16± 0.13 [a]
	Globulin	2.60±0.2 [c]	2.76± 0.14 [b]	2.79± 0.11 [b]	2.81± 0.09 [b]	3.40± 0.13 [a]
	Alb./Glob. ratio	0.57± 0.01 [b]	0.60± 0.03 [b]	0.59± 0.01 [b]	0.60±0.02 [b]	0.68± 0.1 [a]
	ALT	41.20± 3.1 [b]	41.00± 2.2 [b]	43.14± 1.09 [a]	40.90±2.03 [c]	36.97± 3.01 [d]
	AST	38.01± 1.01 [b]	37.45± 1.2 [c]	39.21±2.01 [a]	38.41± 0.97 [b]	29.89± 1.01 [d]

For each day means within the same row carrying different letters are significantly different at ($P < 0.01$)

The total proteins, Albumin and globulines of the experimentally infected *O. niloticus* were decreased when compared with the control group, probably due to the liver damages produced by *E. tarda* infections, hence decreasing the serum protein concentrations (Benli and Yildiz, 2004) and increased the ALT and AST levels. Furthermore, all plasma proteins were synthesized in the liver except gamma globulins which are produced by lymphocytes, which in turn decreased due to lymphopenia (Coles, 1986).

The histopathological changes, (Fig. 13-23); severe congestion of central veins associated with dilated and engorged blood capillaries. Degenerative changes of the hepatocytes with enlarged eosinophilic cytoplasm and fatty

changes. Marked degeneration and necrosis of the pancreatic portions associated with haemosidrin pigment deposition. The kidneys of infected *O. niloticus* showed severe degeneration of the renal tubules with diffuse necrosis .The spleens of the infected *O. niloticus* with *E. tarda* showed severe lymphoid depletion due to immuno-suppression (Pirarat *et al*., 2007), causing extensive apoptosis ((Ibrahem *et al*., 2011) and vacuolation or necrosis of melanomacrophage centers associated with partial to complete loss. These finding were nearly similar to that reported by (Herman and Bullock,1986; Darwish *et al*., 2000; Miwa and Mano, 2000; Sahoo *et al*., 2000; Shaaban, 2007; Vasquez-Piñeros *et al*., 2010 and Ibrahem *et al*., 2011).

The most sever histopathological pictures were found in the experimentally infected group 1, carrying the four virulence genes of *E. tarda*, followed by group 2 then group 3 and less sever in the 4th group; this may be due to the variation in the number of the virulence genes.

The findings of histopathological changes could be referred to the action of the powerful haemolysin of *E. tarda* (Minamia *et al*., 1979). *E. tarda* colonizes extracellular and intracellular in various organs such as liver, spleen, kidney and intestine (Mohanty and Sahoo, 2007).

Also, may be attributed to the potential virulence factors of *E. tarda* such as siderophores, cell adhesion factor and cell invasion activity and two types of hemolysin, dermotoxin, cytotoxin, enterotoxin which induced the necrosis and the degenerative changes in most organs and the hepatic and nephric virulence factors (Chen *et al*., 1996; Miwa and Mona, 2000 and Mathew *et al*., 2001).

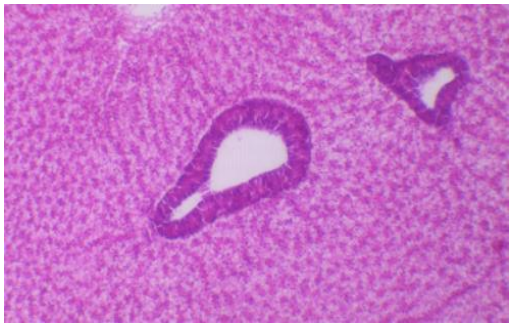

Fig. 13. Control group, Liver of *O. niloticus* showing normal hepatocytes and hepatopancreas, (H&E, X 100).

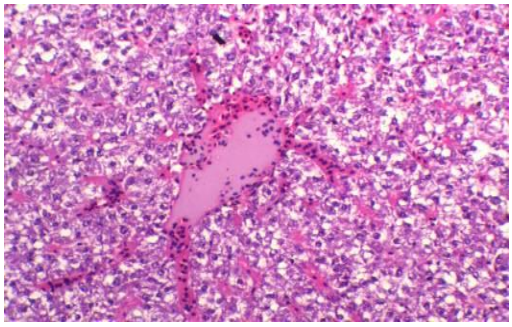

Fig. 14. group (1); Livers of *O. niloticus* experimentally infected with *E. tarda* showing severe congestion of central veins (arrow) associated with dilated and engorged blood capillaries (head arrow), (H&E, X 200).

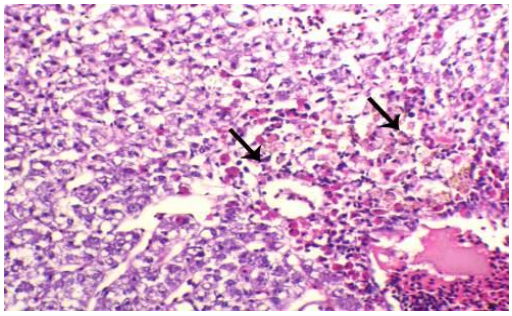

Fig. 15. group (1), Livers of *O. niloticus* experimentally infected with *E. tarda* showing marked degeneration and necrosis of the pancreatic portions associated with haemosidrin pigment deposition (arrow), (H&E, X 200).

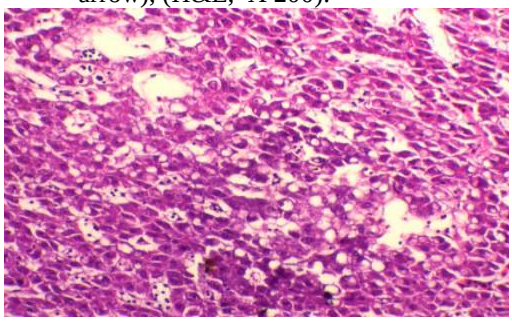

Fig. 16. group (1), Livers of *O. niloticus* experimentally infected with *E. tarda* showing vacuolation and degeneration of hepatocytes associated with atrophy of hepatic cords (arrow) and marked dilatation of blood vessels, (H&E, X 200).

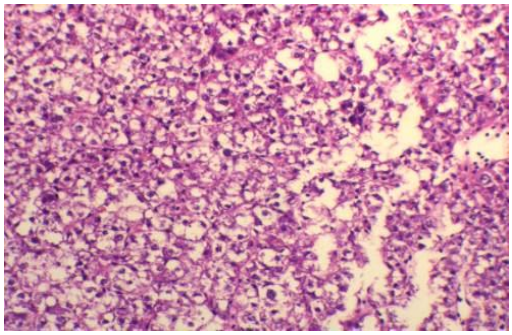

Fig. 17. Group (2); Livers of *O. niloticus* experimentally infected with *E. tarda* showing marked vacuolar degeneration of hepatocytes (arrow), (H&E, X 200).

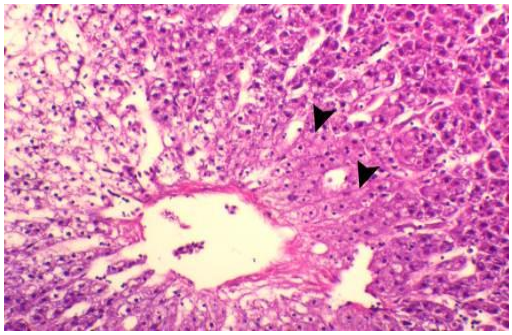

Fig. 18. group (2); Livers of *O. niloticus* experimentally infected with *E. tarda* showing degenerative changes of the hepatocytes with enlarged eosinophilic cytoplasm in the centrolobular areas, (head arrow), (H&E, X 200).

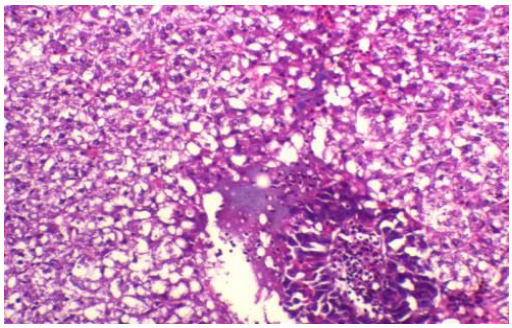

Fig. 19. group (3), Livers of *O. niloticus* experimentally infected with *E. tarda* showing degeneration involving area around the pancreatic portion (arrow), (H&E, X 200).

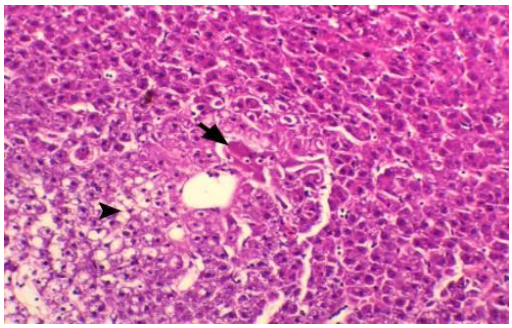

Fig. 20. group (4), Livers of *O. niloticus* experimentally infected with *E. tarda* showing centrolobular necrosis of hepatocytes (arrow) and multifocal vacuolar degeneration (head arrow), (H&E, X 200).

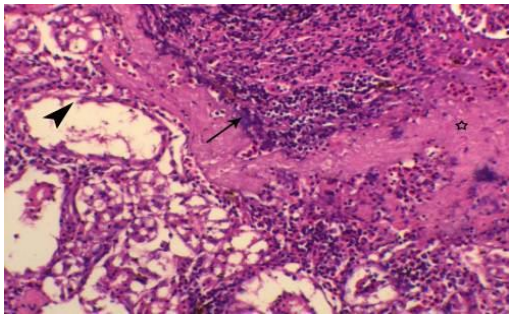

Fig. 21. group (1) , Kidneys of *O. niloticus* experimentally infected with *E. tarda* showing severe tubular degeneration (head arrow) , necrosis of the renal parenchyma accompanied with early stage of calcification and presence of bacterial colonies(arrow), (H&E, X 200).

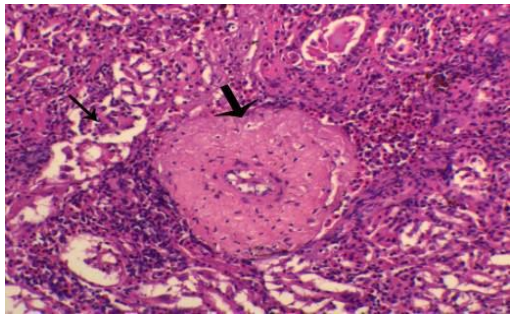

Fig. 22. group (2) , Kidneys of *O. niloticus* experimentally infected with *E. tarda* showing diffuse tubular degeneration (thin arrow) and marked hyalinosis of the blood vessels (thick arrow), (H&E, X 200).

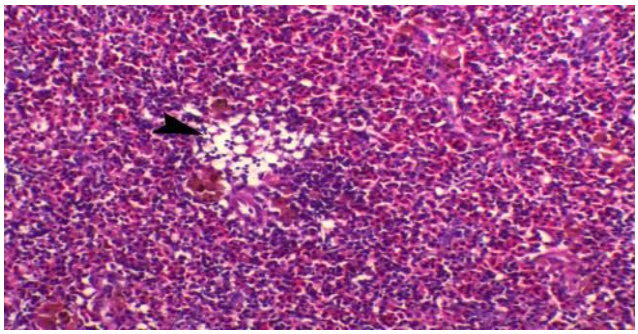

Fig. 23. group (1), spleen of *O. niloticus* experimentally infected with *E. tarda* showing severe lymphoid depletion within white pulp associated with necrosis of melano-macrophage centers (head arrow), (H&E, X 200).

CONCLUSION

Edwardsiellosis has a major economic impact on aquaculture industry under Egyptian conditions, experimentally infected *O. niloticus* with different *E.tarda* strains of *O. niloticus* or *C. gariepinus* origin, expressed clear septicemia and mortalities; *O. niloticus* can get infected with *E. tarda* isolated from another species. There were significant differences in the presence or absence of a number of virulence genes among *E. tarda* strains; describing the

strong congruence of virulence genes and pathogenicity, suggesting that the virulence profile may be useful tool for prediction of pathogenicity.

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تطابق جينات ضراوة الادواردسيلا تاردا المعزوله من البلطي النيلي والقراميط مع قدرتها على الإمراض في البلطي النيلي

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[٢] *قسم بحوث أمراض وصحة الاسماك، المعمل المركزى لبحوث الثروة السمكية، مركز البحوث الزراعية، وزارة الزراعة، مصر.*

الملخص العربى

تعتبر عدوى الادواردسيلا تاردا من امراض الاسماك الخطرة التي تسبب خسائر اقتصادية عالية. أجريت هذه الدراسة بهدف دراسة الجرعه نصف المميته والقدرة على الأمراض لميكروب الادواردسيلا تاردا المعزوله من نوعين مختلفين من الأسماك (البلطي النيلي والقراميط)، ومدى ارتباطها بعدد جينات الضراوة في كل عترة. أظهرت النتائج أن السلالة الأكثر شدة كانت سلالة١ (المجموعة ١) حيث تسببت في وفيات بنسبة ٥٠٪ خلال ١٢ ساعة عند تركيز ١.٨ × $١٠^{٥}$. وقد تم تسجيل العلامات المرضيه التي ظهرت على كل المجموعات. وقد كانت العلامات بارزة بوضوح في المجموعة السمكية الأولى المصابة بسلالة الادواردسيلا تاردا التي تحتوي على الاربعه جينات ضراوه. ومع ذلك، كانت هناك علامات موجودة أيضا في المجموعات الثلاث الأخرى التي تحتوي على ثلاثة، واثنين وجين واحد من جينات الضراوة على التوالي، ولكن بدرجات خفيفة ومتباينه ، في حين لم تظهرعلى المجموعه الضابطه أي تغييرات مرضيه. وقد أجريت بعض الاختبارات الدموية علي دماء الأسماك المعدية بالعترات والضابطة، كما تم تصنيف نسب خلايا الدم البيضاء. أيضا، أجريت بعض الدراسات البيوكيميائيه في مصل دماء الأسماك المعدية بالعترات والضابطة لدراسة بعض الإنزيمات والبروتينات والتي كشفت عن انخفاض في كرات الدم الحمراء،والهيموجلوبين، البروتينات الإجمالية، الألبومين والجلوبيولين وزيادة في تركيز كرات الدم البيضاء وبعض الانزيمات. كما تم تسجيل الدراسات الهستوباثولوجيه علي بعض انسجه الأسماك المعدية والضابطة وتم تسجيل نتائجها.