EVALUATION OF DIFFERENT VACCINATION STRATEGIES FOR CONTROL OF (MAS) IN NILE TILAPIA (*O. NILOTICUS*) IN EGYPT

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Abstract

A highly pathogenic isolate of *Aeromonas hydrophila*, isolate No1, was identified through evaluation of the Crude extra-cellular product and estimation of LD50. Three types of formalized whole culture *Aeromonas hydrophila* vaccine (FWC) were prepared, FWC vaccine alone, FWC vaccine mixed with Freund's complete adjuvant (FCA), and FWC vaccine mixed with Freund's incomplete adjuvant (FIA), tested for sterility and administered to female Nile tilapia (*Oreochromis niloticus)* using two methods of delivery. Micro-agglutination and the double immuno-diffusion tests were performed on serum, mucus and eggs to evaluate maternal immunity. The relative level of protection (RLP) was calculated after challenge infection.

Vaccine safety showed that fish vaccinated with FWC vaccine +FCA expressed severe skin lesions and died. RLP of formalin-inactivated whole culture vaccine alone (S/C or I /M) or incorporated with FIA (I/M) protected 100, 88.8 and 91.1 percent of vaccinated fish, respectively. Subcutaneous administration of the vaccine incorporated with the adjuvants provided partial protection, 66.6%.

Serum and mucus showed increased agglutinins four and six weeks post initial vaccination and up to the 8th week in FWC vaccinated fish and fish subcutaneously vaccinated with FWC+FIA. The agglutinating antibodies from eggs of vaccinated females had an agglutinating titer of 160 compared to 10 in the control.

Double immuno-diffusion revealed that antibodies were traced three and four weeks post initial vaccination with WFC vaccine alone and on the 5th week after vaccination with FWC vaccine +FIA. Antibodies were detected on the 4th week in mucus after subcutaneous vaccination. Total protein in mucus increased in the vaccinated fish. Total protein was 3 and 2.1 g/dL in eggs of vaccinated fish and the control, respectively.

We concluded that the prepared monovalent formalized whole culture vaccine alone or incorporated with FIA adequately protected tilapia against the homologous virulent isolate. Presence of antibodies in egg samples increased the possibility of maternal transfer of immunity.

INTRODUCTION

The Motile Aeromonas Septicemia (MAS), caused by *Aeromonas hydrophila* is among the dangerous diseases encountered in freshwater fish culture, (Groff and Lapatra, 2000 and Karunasagar *et al*, 2003). Motile aeromonads are a heterogeneous group of organisms which are involved in a number of diseases of warm water fish. They are commonly associated with bacterial hemorrhagic septicemia, infectious dropsy, red mouth disease, and ulcerative conditions. A variety of factors have been associated with virulence of the bacteria including hemolysins, proteases, surface array proteins and acetylcholinesterase (Karunsagar *et al.*, 1997).

Nile tilapia, dubbed the “aquatic chicken” (Maclean 1984) is one of the most important cultured fish species in many parts of the world including Egypt (Davlin, 1991, Pullin, 1997). Since tilapia is a top priority fish in the tropics, the need to evaluate strategies for improving fish health and immunity is a must.

The extensive use of chemotherapeutic agents to control MAS may become an ecological threat. It increases the selective pressure on microbial agents and therefore encourages the emergence of bacterial resistance, it is not recommended for the treatment of toxin producing bacteria, e.g., *Aeromonas hydrophila* (Janda 1991) since it affects the bacteria but not the toxin, and it creates a public health hazard if the flesh of treated fish is used for human consumption (Flores *et al*, 2003).

Vaccinations are identified as active immunization that results in the increase in the concentration of naturally acquired antibodies (Schaperclaus 1972). The efficiency of vaccination is largely dependant on the immune status of the fish and the conditions under which the fish were kept (Robohm and Koch, 1995).

Immunization against *Aeromonas hydrophila* is difficult because of its heterogeneity as well as its stereotyping. To cope with this problem, several researchers planned to produce different types of vaccine such as the formalized whole culture vaccine (Gado 1995), the hyper- osmotic infiltration vaccine (AQUIGRUP 1980), the toxoids (Baba *et al.,* 1988) and the genetically engineered live bacteria with removal of one of the aerolysin genes (virulence gene), (Soliman *et al.,* 1989). Successful vaccination programs were ones targeted against the *Aeromonas hydrophila* isolates endemic to particular areas, i.e., vaccines (AQUIGRUP 1980).

A properly functioning immune system is critical in maintaining the fitness and health of an organism. Therefore, disease challenge studies are important tools for examining the health status, performance, and immunity. This technique provides an opportunity to determine the effect of exposure to xenobiotic (bacteria) on the performance and immunity of the fish species and on its natural habitats (Arakoosh *et al*., 2005).

This work aims to compare and evaluate different vaccination strategies for control of MAS in Nile tilapia (*O. niloticus).*

MATERIALS AND METHODS

Fish

A total of 740 female Nile tilapia (*O. niloticus)* were obtained from an intensive fish farm at Kalyoubia Governorate, Egypt. The fish appeared healthy. The fish were transferred and maintained for acclimatization in glass aquaria supplied with dechlorinated tap water at 25 ± 1°C and aeration in the wet laboratory, Dept. of Fish Disease and Management, Faculty of Veterinary Medicine, Cairo University.

Bacterial isolate

Virulent *Aremonas hydrophila*, isolate No. 1, was locally isolated from clinically diseased Nile *tilapia* and identified. The isolate was previously tested for its pathogenicity and found to be highly pathogenic; the isolate was supplied by Mai D. ibrahem, Department of fish disease and management, Cairo University.

Evaluation of the Crude extra-cellular product of *Aeromonas hydrophila* No 1

The preparation of the Crude extra-cellular product was carried out according to Branden and Janda (1987). 0.2 ml of the filtrate was injected intramuscular in 10 Nile tilapia females as reported by Allan and Stevenson (1981). Fish were kept under observation for 3 days and mortality was monitored.

Determination of virulence (LD50)

A total of 130 Nile tilapia, (*O. niloticus*) were used for testing the degree of virulence (LD50) of the selected isolate according to the method described by Paget and Burners (1964). 10 fish were injected using the same procedure with PBS (PH 7.2) [control (1)], other 10 fish were held untreated [control (2)]. The observation time was 7 days**.** The degree of virulence (LD50) was the dilution resulted in 50% mortalities.

Preparation of monovalent *A. hydrophila* vaccine

Preparation of the vaccine was performed according to (Gado 1995) with modifications. One colony from *Aeromonas hydrophila* isolate No 1 was inoculated in Trypticase Soya Broth and incubated at 30°C for 24 hr in a rotary shaker incubator at 200 oscillations/minute. The broth culture flask was checked for purity and the total colony count was adjusted to 2.4×10^8 C.F.U/ ml. for inactivation using formalin (37%), the flask was placed on a magnetic stirrer at a medium speed and a temperature of 37°C. Formalin was added by dripping into the broth culture to a final concentration of 2% V/V. The flask was left overnight in the lamina flow. Three types of vaccine were prepared, sealed, and kept in a refrigerator. The types of vaccine used were:

- Formalized whole culture vaccine, prepared according to Soliman *et al.,* (1991)
- Formalized whole culture vaccine mixed with Freund's complete adjuvant.(FWC vaccine +FCA), prepared upon usage according to Abdel Fatah (1991)
- Formalized whole culture vaccine mixed with Freund's incomplete adjuvant. (FWC vaccine +FIA), prepared upon usage according to Boesen *et al*., (1997)

Evaluation of monovalent *A. hydrophila* vaccine

The vaccine was evaluated using the protocol of the British Veterinary Codex (1970) in Tatner, M.F. 1993 as follows:

Sterility test

The prepared vaccine was tested for sterility from bacterial contamination using Tryptic Soya Agar (Gibco), Brain Heart Infusion Agar (BioTeC), blood agar media, and MacConky Agar (BioTeC). Testing for Mycotic infection and Mycoplasma was conducted using Sabaroud Dextrose Agar and Mycoplasma Selective Media + supplement (Oxoid) 10215.

Safety test

Eleven groups of *O. niloticus*, 30 individuals each, were used in the safety test experiments as shown in Table (1)

Potency test

Based on the results of the safety test, the FWC vaccine +FCA groups were excluded. Four groups each of 50 *O. niloticus* in addition to 2 control groups each of 25 were used for potency test as shown in Table (2). Each of the 4 groups was vaccinated using initial and booster dose of one type of the prepared vaccine with 2 weeks interval. All fish were observed for 8 weeks post initial vaccination.

Table 1. safety test

Group number	No. of fish	Dose/ ml*	Route	Type of the vaccine
1	30	0.2	I/M	FWC vaccine +FCA
2	30	0.2	S/C	FWC vaccine +FCA
3	30	0.2	I/M	FWC vaccine +FIA
4	30	0.2	S/C	FWC vaccine +FIA
5	30	0.2	I/M	FWC vaccine alone
6	30	0.2	S/C	FWC vaccine alone
7	30	0.2	I/M	Saline+ FCA
8	30	0.2	S/C	Saline+ FCA
9	30	0.2	I/M	Saline+ FIA
10	30	0.2	S/C	Saline+ FIA
11	30	0.2	I/M	Saline only
Total	330	-	-	-

*****Containing approximately 2.4 $\times 10^8$ CFU/ml.

Table 2. Potency test:

Group	No. of fish	Dose*/ml	Route	Type of the vaccine
1	50	0.2	I/M	FWC vaccine +FIA
2	50	0.2	S/C	FWC vaccine +FIA
3	50	0.1	I/M	FWC vaccine
4	50	0.1	S/C	FWC vaccine
5	25	0.2	I/M	Saline only
6	25	0.2	S/C	Saline only
Total	250	-	-	-

* contain 2.4 $\times 10^8$ CFU/ml.

Sampling and biological materials

Sampling

Fish under experimentation were anesthetized using tricaine methane sulphonate (MS222). Blood samples were collected from the caudal vein of vaccinated and control groups using sterile dry test tubes as has been reported by Austin and Austin (1999). Serum samples were collected at the beginning of the experiments and weekly up to 8 weeks post initial vaccination, sera were collected and stored at -20^{o}C until used. The mucus layer was gently scrapped off from the fish surface by means of a clean sterile glass slide as described by Krovacek *et al.,* (1987). Mucus samples were collected and stored at -20^{o}C until used.

Unfertilized eggs were collected from vaccinated and control fish using the method of Swain *et al.*, (2006). Egg samples were collected by the end of the 8th week post initial vaccination as well as from the control fish and stored at -20^{o}C until used. sggs were placed in a sterile syringe and pushed through its nozzle several times to ensure its rupture and emulsification.

Preparation of hyper immune serum

Hyper immune serum against *Aeromonas hydrophila* (isolate No 1) was prepared in New Zealand rabbits according to Soliman *et al.,* (1988). Sera were collected and stored at -20^{o}C until used. The sera were used as positive control in serological tests.

Preparation of the antigen of *Aeromonas hydrophila* (isolate No 1) used for The Micro-agglutination test

Formalized whole culture vaccine was used as antigen in the serological tests.

Antigen preparation for double immunodiffusion test

The soluble antigen was prepared according to Leblanc *et.al,* (1981).

Serological and biochemical tests

Total protein concentration

Total protein concentration was determined in each mucus and egg sample according to (Weicheselbaun, 1964).

The Micro-agglutination test

Serum and mucus samples were subjected to the micro-agglutination test according to Hay *et al.*, (2002).

Double immunodiffusion test

Serum, mucus and egg samples were subjected to the Double immunodiffusion test, according to Ouchterloney (1962).

The challenge infection

The vaccinated and the control fish were injected intra-peritoneally with 0.2 ml of 24 hr broth culture of *Aeromonas hydrophila* isolate No 1 containing approximately 10^9 CFU/ml^{-1} according to (Moselhy *et al.*, 2008). Fish mortality and development of clinical signs were recorded for 10 days post challenge. The RLP was calculated using the following equation after Amend (1981) as follows:

(R.L.P.) = 100 - % of mortality of vaccinated fish ÷ % mortality of control × 100

RESULTS AND DISCUSSION

The results of the inoculation of the crude extra-cellular product of *Aeromonas hydrophila* isolate No (1) proved to be pathogenic, as the injection of the crude extra - cellular products in the experimental fish resulted in external muscle lesion manifested by swelling, slight redness with development of lequifactive necrosis, muscular ulcer and mortality of the inoculated fish. Lesions resembled those associated with injection of whole culture of *A. hydrophila* photo (1). Our results are in agreement with those of Allan and Stevenson (1981) and Kinya and Takagi (1986). They found that the toxins produced by *A. hydrophila* have a dermonecrotic effect on the muscles of injected fish at the site of inoculation which is characterized by edema, redness and muscular necrosis expressed as ulcers. They added that the same lesion was produced due to injection of *A. hydrophila* whole culture, and concluded that the extra-cellular products are one of the major virulence factors in *A. hydrophila* bacteria and can be responsible for development of the disease.

Based on the LD50 of the selected *A. hydrophila* isolate No.1, the selected isolate was considered virulent according to the classification of Santos *et al,* (1988), and Soliman *et al.*, (1989) who reported that LD_{50} of (10^4 to 10^5) was considered in the virulent category whereas (10^6 to 10^7) was classified as weakly virulent and over 10^8 were avirulent isolates. From the previous results, *Aeromonas hydrophila* isolate No1 was chosen for vaccine preparation.

Results presented in Table (3) (Plate 2 and 3) shows that all fish groups (2 groups) vaccinated with the formalized whole culture *A. hydrophila* vaccine incorporated with (FCA) showed severe skin lesions and mortality, moreover، the 2 control groups received the (FCA) with saline and injected either I/M or S/C showed the same criteria. This indicates that (FCA) had a severe harmful effect on tilapia when injected either intramuscularly or subcutaneously, this finding is supported by the results of Press and Llilehaugh, (1995) who stated that oil vaccine can cause some local inflammatory reactions. Harlow and Lane (1988), Smith (1990) also stated that one of the disadvantages of (FCA) is the difficulty of mixing with immunogen and, thus, it can cause tissue necrosis at injection site. This is different from reports by Harrell *et al.*,

(1975), who vaccinated rainbow trout against *Vibrio anguillarum* with a bacterin incorporated with Freund's complete adjuvant (FCA) I/P, and Thune and Plumb (1982) who concluded that I/M injection of 0.1 ml of *A. hydrophila* bacterin suspended in equal volume of (FCA) in catfish was not harmful. Saeed (1983) also vaccinated channel catfish against *E. ictaluri* using I/P injection with bacteria + (FCA). Abdel Fatah (1991) also reported vaccination of Nile Tilapia with I/M injected *A. hydrophila* emulsified either in FCA or FIA and Yin *et al.*, (1996) vaccinated channel catfish against *A. hydrophila* intraperitoneally. It is possible that the S/C or I/M injections are not the suitable routes for administration of FCA incorporated with vaccines and/or that *O. niloticus* is sensitive to such adjuvant. Therefore, both groups receiving FCA with the vaccine were excluded.

The criteria used for evaluating the degree of protection conferred to the recipients are judged by the relative level of protection of the different vaccine preparations, development of clinical signs following intramuscular challenge with the virulent homologous *A. hydrophila* virulent isolate, re-isolation from moribund and dead fish serum, surface mucus and egg agglutination titers as well as antibodies in the precipitation test.

From the data presented in Table (4) and Chart (1), it is obvious that the formalin inactivated whole culture vaccine either alone (by S/C or I /M routes) or incorporated with FIA by (I/M) route protected 100, 88.8 and 91.1 percent of vaccinated fish respectively. While the vaccine incorporated with the adjuvant and given subcutaneously partially protected fish (66.6%) against challenge. These findings are in agreement with Thune and Plumb (1982) who stated that the subcutaneous injection was a superior method of vaccination to immersion regardless of the antigen preparation in channel catfish. However, our findings differ from that of Tiecco *et al.*, (1988) after working on vaccination of eels by *A. hydrophila* who found that intramuscular injection and by immersion gave significant degrees of protection.

There are many conflicting reports regarding the protective role of fish specific agglutination antibody. Some indicated that there is no correlation between protection and level of serum specific antibodies (McCarthy *et al.,* (1983) and Baba *et al.,* (1988)) .Others reported that these antibodies play an important role in protecting fish against some infections (Eurell *et al.*, (1978), Badran, (1991), Gado, (1994), Viola, (1995) and Yin *et al.*, (1996). Results presented in Table (5) showed gradual rise of the serum agglutination titre in all vaccinated groups to reach a maximum of 160 -1280 by 4 weeks after the initial vaccination followed by a gradual decline until the 8th week where it reached 40 in groups receiving the vaccine alone I/M and 80 in other groups.

It is worthy to mention that no antibodies could be detected in the sera of control fish throughout the experimental period. These results indicate the validity of the test in detecting antibodies to *A. hydrophila*, and come in accordance with those of Eurell *et al.*, (1978) who found the test to be effective in detecting the level of serum

agglutinins to *A. hydrophila* in channel catfish, Soliman *et al.*, (1989) who detected serum agglutinating antibodies against *A. hydrophila* in the serum of *O. niloticus* one week post vaccination reaching a peak 4 weeks post immunization, Gado, (1994) who used the test to estimate the agglutinating antibody following immunization of *Clarias lazera* with formalized *A. hydrophila* bacterin, Viola, (1995) who recorded gradual rise of the agglutination titre in *O. niloticus* vaccinated with formalin inactivated bacterin of *A. hydrophila*, and Yin *et al.*, (1996) who found that vaccinated catfish displayed humoral antibodies from the 7th day which peaked on the 28th day post vaccination with formalin inactivated *A. hydrophila* vaccine.

It has been established that the surface of fish is covered with a mucus layer, varying in thickness and forming a barrier to pathogens, (Yin *et al.*, 1996) and Dorson (1981) showed that immunoglobulins in this secretion had a weak agglutinating activity. Therefore, it is logic to suggest that the specific antibody in the mucus might play an important role in preventing *A. hydrophila* from attacking and penetrating the epithelium of gills, and skin of fish.

Agglutinating titers in the body surface mucus are presented in Table (6). The maximum titer was obtained on the 4th week post initial vaccination in all injected groups (160 - 320). The control group showed a titer of 10 during the whole experimental period. The increase in the total protein and in agglutinins of the mucus as a response to vaccination in the surface mucus samples during the 4th and 6th weeks post initial vaccination (Table 6). This increase extended up to the 8th week in fish vaccinated with FWC alone and fish receiving FWC + FIA vaccine subcutaneously also as a response to vaccination. This, in addition to the failure of isolation of *A. hydrophila* from the skin of vaccinated fish is in agreement with the reports of Dorson, (1981), Badran (1991) and Yin *et al.*, (1996) and may throw some light on the protective role of the surface mucus immunoglobulin in inhibiting the growth of the organism on the surface of the body.

In a preliminary study the agglutinating antibodies specific for *A. hydrophila* could be recovered from unfertilized egg material of mature female tilapia. The agglutinating titer was 160 in vaccinated fish compared to 10 in control fish (Table 7). Moreover, the total protein in eggs from vaccinated fish was 3 g/dL compared to 2.1 in control fish. These results suggest the transfer of antibodies into eggs of Tilapia and consequently their progeny. These antibodies in eggs can protect fry from infection and fish may remain healthy as long as this passive immunity remains active. This is especially important since the lack of immune competence in the early stages of life may to severe mortality in larval stages of different fish species (Swain *et al.*, 2006). A similar suggestion was reported by Scott *el al.,* (1989). Swain *et al.,* (2006) investigated the passive transfer of maternal antibodies following immunization with a virulent *A. hydrophila* bacterin through agglutination test which revealed a significant increase in specific serum antibody response in the brood fish of Indian major carp, *Labeo rohita* (Ham.) following immunization with a virulent *Aeromonas hydrophila*

bacterin 1 month prior to breeding. This immunity was transferred to larvae through the egg. The present study indicated the role of maternally derived antibody in the protection of hatchlings of tilapia against specific pathogens. However, this point still is in need for further investigation.

Results Concerning the double immunodiffusion tests, revealed that antibodies could be traced in sera of all vaccinated fish on the 3rd and 4th weeks post initial vaccination only, and in sera of fish vaccinated with FWC + FIA vaccine on the 5th week (Tables 5 and 6). Moreover, it could be detected on the 4th week in mucus samples of fish that received the vaccine by injection. It seems that they appear late and stay for a short period in serum and mucus samples. It is worthy to mention that none of the control fish showed positive reaction to the test.

It could be concluded from the present investigation that the prepared monovalent formalized whole culture vaccine alone or incorporated with FIA offered a good immunity against the homologous virulent isolate. The presented serological data and the results of the relative level of protection (RLP) confirm the role of the humoral antibodies in protecting fish against *A. hydrophila* infection. Presence of antibodies in eggs strengthens the possibility of maternal transfer of immunity and supports the results of the agglutination test.

Table 3. treatments and subsequent effects on Nile tilapia, *Oreochromis niloticus*.

Type of vaccine	Percent of dead fish	The observed clinical abnormalities
FWC vaccine +FCA	100%	All fish died with severe ulceration at the site of inoculation
FWC vaccine +FCA	100%	All fish showed skin lesion at the site of inoculation with swimming abnormalities
FWC vaccine +FIA	20%	The fish showed darkness in skin + swimming abnormalities, off food and signs of asphyxia
FWC vaccine +FIA	26.6%	The fish showed darkness in skin + swimming abnormalities.
FWC vaccine alone	6.6%	The dead fish showed no clinical abnormalities.
FWC vaccine alone	0%	Fish in very good condition
saline +FCA (control)	100%	All fish showed skin lesion at the site of inoculation
saline +FCA (control)	100%	All fish showed skin lesion at the site of inoculation
saline +FIA (control)	33.3%	The fish showed signs of swimming abnormalities and in bad condition
saline +FIA (control)	26.6%	The fish showed signs of swimming abnormalities and in bad condition
Saline only	0%	Fish in very good condition

Table 4. Comparison between the relative levels of protection (RLP) afforded by the different types of *Aeromonas hydrophila* vaccines of *O. niloticus.*

Type of vaccine	Results*	Percent of survivals	RLP
FWC vaccine +FIA I/M	4/50	92	91.1
FWC vaccine +FIA S/C	15/50	70	66.6
FWC vaccine I/M	5/50	90	88.8
FWC vaccine S/C	0/50	100	100
control	45/50	10	-

*Number of dead fish/number of inoculated fish.

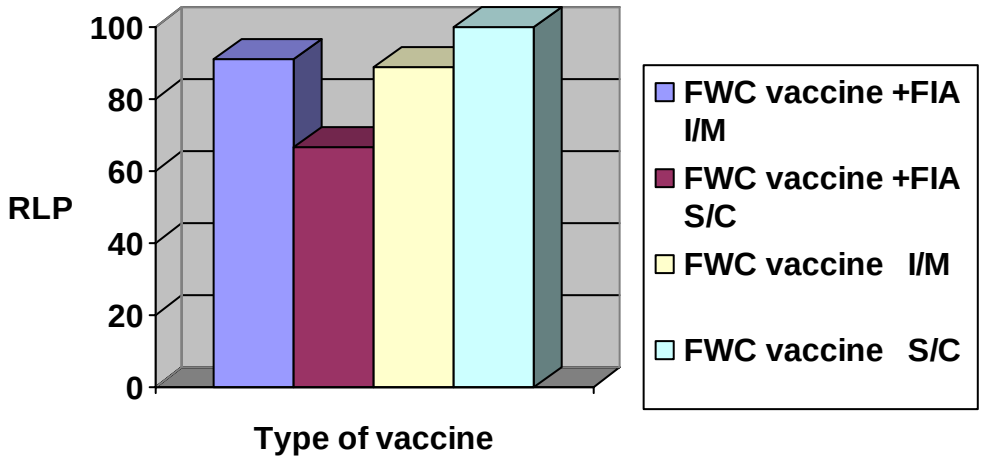


Chart 1. Relative levels of protection associated with Vaccination of Nile tilapia, *O. niloticus*, against *A. hydrophila*

Table 5. results of agglutination test and double immunodiffusion test in the serum of vaccinated and control fish.

Time/ week	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Test Type of vaccine	Agglutination titer								Double immuno-diffusion							
FWC vaccine +FIA (I/M)	-	80	320	160	160	160	80	80	-	-	+	+	+	-	-	-
FWC vaccine +FIA (S/C)	-	40	160	160	160	160	80	80	-	-	+	+	+	-	-	-
FWC vaccine (I/M)	-	40	640	160	160	160	80	40	-	-	+	+	-	-	-	-
FWC vaccine (S/C)	-	40	320	1280	640	640	160	80	-	-	+	+	-	-	-	-
Control - ve	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control +ve	2560								+							

Zero time : the results are negative in both tests in all groups of vaccinated and control fish .

Table 6. Results of the mucus agglutination test of vaccinated and control fish.

Time/week	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8
Test Type of vaccine	Agglutination titre					Double immunodiffusion test					Total protein (g/dl)				
FWC vaccine +FIA (I/M)	10	160	160	160	160	-	-	+	-	-	2.5	2.4	2.7	2.7	2.5
FWC vaccine +FIA (S/C)	10	160	320	160	160	-	-	+	-	-	2.5	2.3	2.8	2.7	2.8
FWC vaccine (I/M)	10	80	160	160	80	-	-	+	-	-	2.5	2.5	2.8	2.8	2.8
FWC vaccine (S/C)	10	160	320	160	160	-	-	+	-	-	2.5	2.4	2.8	3.0	3.0
Control fish mucus	10	10	10	10	10	-	-	-	-	-	2.5	2.5	2.5	2.5	2.5

Table 7. Results of the micro-agglutination titer, double immunodiffusion test, and total protein, g/dl, in egg samples.

Sample Test	Eggs from vaccinated fish	Eggs from control fish
Micro -agglutination titer	160	10
Double immunodiffusion test	+	-
Total protein (g/dl)	3	2.1

Photo 1. *O. niloticus* showing deep necrotizing ulcers at the site of injection of extra - cellular products of *A. hydrophila* with liquefactive necrosis surrounded by slight zone of redness.

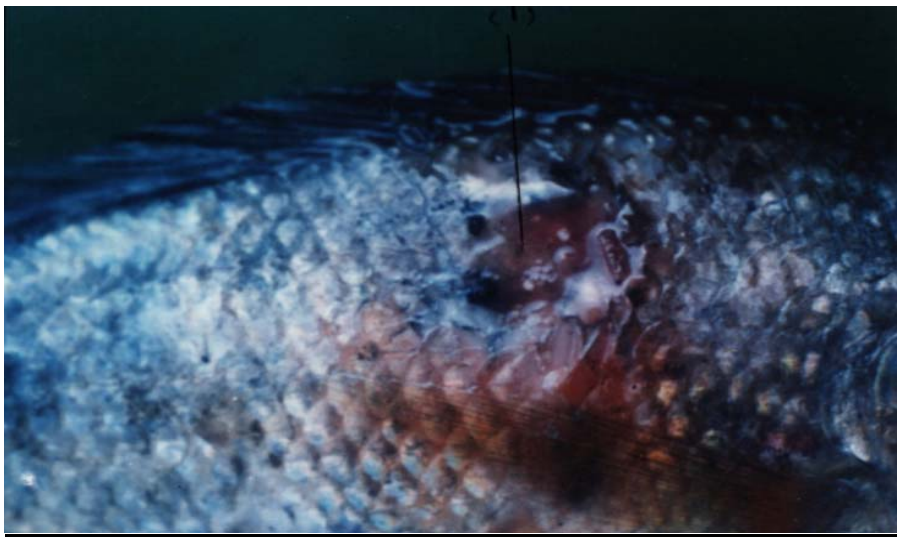
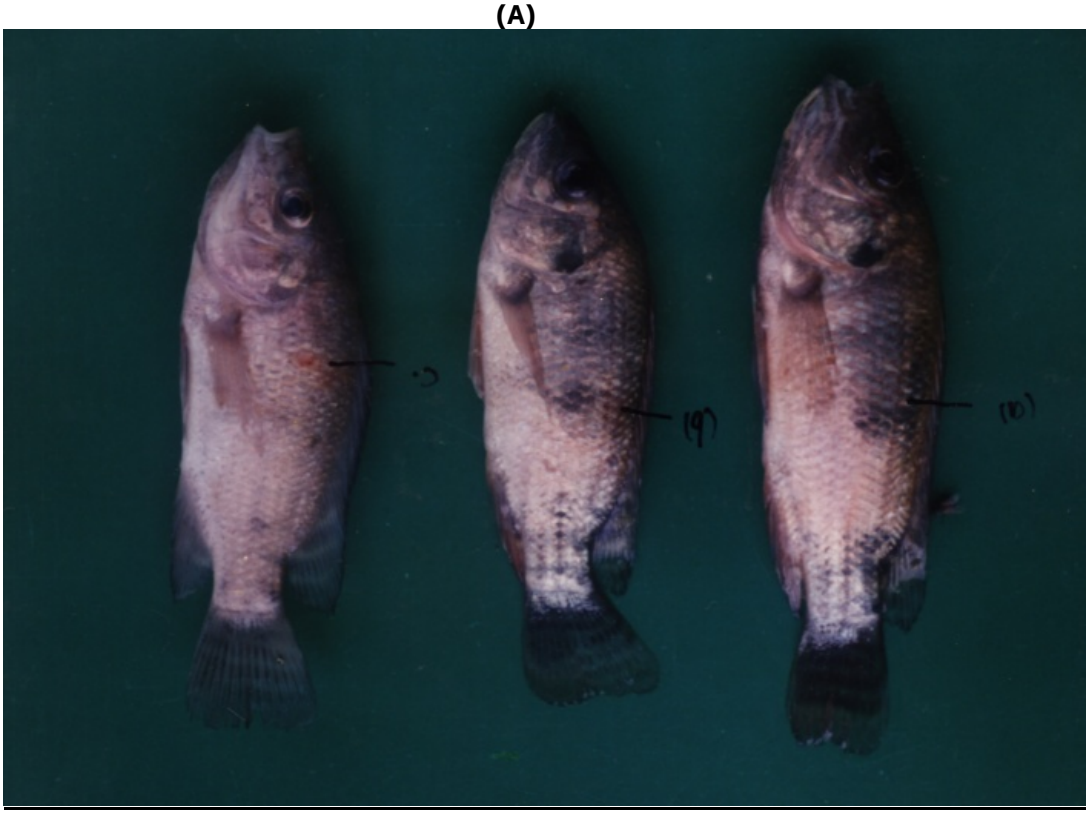


Photo 2. *O. niloticus* showing abscess formation as a result of FCA + vaccine inoculated by S/C or I/M routes.

(A)



(B)

Photo 3. (A,B) *O. niloticus* showing severe abscess formation at site of inoculation follow the use of FCA + vaccine by S/C or I/M routes.

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تقييم لإستراتيجية الأنواع المختلفة من اللقاحات للتحكم بمرض التسمم الدموى الايروموناسى فى البلطى النيلى فى مصر

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تم تحديد عتره شديدة الضراوة من ميكروب الايروموناس هيدروفيلا باستخدام تقييم المستخلصات الخارجية الخام وLD . تم تحضير ثلاثة أنواع من اللقاحات الميتة بواسطة الفورمالين الأولى و المحضرة بدون اى أضافات الثانية مضاف على اللقاح مستحلب فرويد الزيتي الكامل الثالث مضاف إليه مستحلب فرويد الزيتي غير الكامل. كل أنواع اللقاحات المحضرة تم اختبارها للنقاء و السلامة و الضراوة باستخدام إناث اسماك البلطي و طريقتين للتطعيم ، لتقييم الضراوة تم إجراء اختبارات الأجسام المناعية المناسبة على عينات المصل و المخاط الجلدي و البيض لاختبار مناعة البيض من الامهات المحصنة. تم حساب معدل رفض العدوى بعد إجراء العدوى الصناعية.

نتج عن اختبار السلامة للقاح مدى سمية استخدام اللقاح المحضر باستخدام مستحلب فرويد الزيتي الكامل زذلك بظهور الاعراض المرضية على الجلد والوفيات. و بحساب معدل رفض العدوى بعد إجراء العدوى الصناعية وجد ان استخدام مستحلب فرويد الزيتي غير الكامل أعطى اعلي مناعة وصد العدوى الصناعية . تم الخلاصة الى ان تحضير اللقاحات الميتة بواسطة الفورمالين لوحدها او باستخدام مستحلب فرويد الزيتي غير الكامل صد العدوى وزاد مقاومة إناث اسماك البلطي للعدوى باستخدام الميكروب الضارى المحضر منه اللقاح وان وجود أحسام مناعية في بيض الأمهات المحصنة يزيد فرصة مقاومة الزريعة للعدوى بالميكروب فى أوائل ايامها.