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A TRIAL FOR EVALUATION OF LOCALLY POLYVALENT MOTILE AEROMONAD VACCINES IN OREOCHROMIS NILOTICUS AGAINST MOTILE AEROMONAS SEPTICEMIA

By

VIOLA. H. ZAKI*

**Dept. of Avian and Aquatic Animal Medicine, Faculty of Vetrinary Medicine, Alexandria University*

Key Words :

ABSTRACT

*Three groups (60 fishes each) of **Oreochromis niloticus** were used to investigate the efficacy of oral and intraperitoneal vaccination with a polyvalent vaccine prepared from **A. hydrophila**, **A. sobria** and **A. veronii** against motile aeroinonas septicemia. Group (1) was fed with the bacterian of a ratio of 200 mg wet cell/kg fish body weight for 12 weeks dry after day at a ratio of 5% feed of fish body eight. Group (2) was intraperitoneally (i/p) vaccinated with 0.1 ml containing $9x10^9$ cells/ml of the vaccine preparation at zero day and another booster dose at 8^{th} weeks. Group (3) remained as control.*

*Fish immunized either orally or intraperitoneally had a high level of serum antibodies against A. hydrophita. **A. sobria** and **A. veronii** if compared with controls throughout the experimental period (12 weeks).*

*At the beginning of the 4^{th}, 8^{th} and 12^{th} weeks of the experiment; 10 fish from each group were collected, 5 fish were challenged with 0.1 ml containing $7x10^7$ cells of virulent strain of **A. hydrophila** and another 5 fish were i/p challenged with 0.1 contain $7x10^7$ cells of hot strain of **A. veronii**.*

*After challenge with **A. hydrophila**, or **A. veronii**, both groups of immunized fish (RLP) started at the 4th week of the experiment and extended up to 12 weeks.*

*Re-isolation and identification of **A. hydrophila** were done from the head kidney of challenged fish with **A. hydrophila** and **A. veronii**.*

INTRODUCTION

Diseases caused by motile *Aeromonad* strains are of increasing importance worldwide and prospects for their control by vaccination need to be considered.

In Egypt aquaculture, commercial Motile *Aeromonad Septicemia* vaccine have not been licensed, so the development of an effective field applicable vaccine against MAS is of a great economic importance as such disease is the most serious disease of fresh water specially those under stresses as physical handling, overcrowding, water quality deterioration and changes in ambient temperature.

Several laboratory techniques have been successfully used for fish vaccination against MAS. Such techniques included injection (lamer's ***et al.***, 1985- Ruangpan ***et al***, 1986-Badran1987-, Gado 1994) and immersion (Lamers ***et al.***, 1985-Bada ***et al.***, 1988-) aerosol spray (Thune and plumb, 1982) and oral (Badran 1991 and Zaki 1995).

With the intensification of cultured methods for Tilapia species during recent years, it has become necessary to look for a suitable technique for mass administration to fish of all sizes, without stress on the fish. Therefore, oral vaccination is the only suitable method for Egyptian pond rearing of fish (Badran 1991 and Zaki 1995).

The aim of present study was a trial to evaluate the efficacy of a polyvalent motile Aeromonad vaccine developed in our laboratory in protection of Nile tilapia (***Oreochromis niloticus***), against motile Aeromonad Septicaemia, and to correlate the humoral immunity induced with the level of protection observed.

MATERIALS AND METHODS

A-Fish

One hundred and eighty ***Oreochromis niloticus*** with an average weight of 13.5 gm were reared in glass aquaria supplied with dechlorinated tap water and good aeration.

The fish where kept for 14 days as acclimatization period at a water temperature of 20 ± 1°C and fed on commercial fish at a ratio of 15% of their body weight.

B-Bacteria

Three *motile Oreochromis* were used in the present study.

1-Aeromonas veronii, hybridization group 10.

The isolate was isolated and identified by Zaki (1991) and proved to be pathogenic for red tilapia by intermuscular inoculation of 0.1ml of a suspension containing 7x107 viable cells/fish. (Zaki, 1991).

2- Aeromonas sobria hybridization group 8.

The isolate was isolated from diseased catfish and identified by (Zaki, 1991). Serologically, it reacts positively with 0.11 and 0.25 antisera and proved to be pathogenic for red tilapia by intermuscular inoculation of 0.1ml of a suspension containing 7x107 viable cells/fish

3- Aeromonas hydrophila hybridization group 1.

It was isolated from diseased catfish and identified by (Zaki, 1991), and reacted positively with 0.11 anti sera. The isolate proved to be pathogenic for red tilapia by 1/m. Inoculation 7x107 cells/fish (Zaki, 1991).

C-Vaccines and immunization

Formaldehyde (37%) was added to 24 hours broth culture of ***A. hydrophila***, A. Sobria, and ***A. veronii*** to a final concentration of 0.5%. After overnight incubation at 25c, the inactivated cells were harvested by centrifugation (3000rpm for 15 minute) and washed twice in sterile phosphate buffer saline. The sterility and safety of each prepared bacterium was tested according to Ward (1982).

For interperitoneal route of vaccination, an equal volume from each bacterian preparation was adjusted to Mac. Farland No, 3 to contain approximately $9x10^9$ cells/ml and thoroughly mixed. Equal volumes of Freunds incomplete adjuvant and bacterian were well mixed and then considered fit for l/p vaccination.

For the oral route of vaccination, the vaccine was established according to the procedure of Badran (1991). Since the wet-packed whole cell bacterins from the three isolates were mixed with an equal weights, and emulsified in egg yolk at a ratio of 1 gm wet cells/2ml of egg yolk. The emulsified vaccine was mixed with the fish diet at a dose of 200-mg wet cells/kg fish body weight (Badran. 1991).

IMMNUNIZATION PROCEDURES:

After acclimatization period, experimental ***Oreochromis niloticus*** were divided as follow:

Group # 1 60 fish were fed on fish diet + vaccine for 12 weeks at ratio of 5% of their weight day after day.

Group # 2 60 fish were fed on fish diet without vaccine and were vaccinated intraperitoneally with 0.1 ml of the vaccine preparation at zero day and with another booster dose at the 8^{th} week.

Group # 3 60 fish, served as a control, were fed on fish diet without vaccine for 12 weeks and intraperitoneally injected with 0.1ml of sterile saline.

Each group was such divided into 4 replicates ~~each has 15 fish~~ and were kept in a separate glass aquarium during the experimental period.

Blood samples were collected from the caudal vein of the control and the immunized fish at zero day, 4^{th}, 8^{th}, and 12^{th} weeks of the experiment. Separation of serum and determination of agglutinating antibody titters against ***A. hydrophila***, ***A. sobria*** and ***A. veronii*** were done according to Lied ***et al.***, (1975).

Ten fish from each treatment (oral vaccinated, I/p vaccinated and control fish) were collected at zero day, 4th, 8th and12th weeks of the experiment, and were divided into two groups (each have five fish). One group was challenged intraperitoneally with 0.1/ml of sterile saline containing approximately $7x10^7$ cells of *A. **hydrophila*** serogroup 0.11 and the other group was challenged intraperitoneally with 0.1ml of sterile saline containing approximately $7x10^7$ cells of *A. **veronii***. There was no enough numbers of experimental fish for challenge with A. Sobria.

The challenged fish were put under observation for 10 days and the dead fish were collected for re-isolation for the challenged bacteria from the dead kidney on RS medium. Identification of the re-isolated bacteria (either *A. **hydrophila*** or *A. **veronii***) was done according to Aero Key of carnahan (1990) cited by Zaki (1991).

The relative level of protection (RLP) in each group was determined according to Newman and Mainarichs (1982) using the following equation:

$$RLP = \left(1 - \frac{\text{\% of mortalitv in immunized fish}}{\text{\% of mortality in control fish}}\right) \times 100$$

RESULTS

I- Antibody titters

a) Fish immunized either orally or intraperitoneally with *A. **hydrophila***, *A. **veronii***, and *A. **sobria*** bacterian had a high level of serum antibodies when compared with those injected with saline as shown in fig. (1,2). The titter of agglutinating antibodies in the sera from fish immunized orally against *A. **hydrophila*** was 1,3,6 and 7 while, it was 0,2,4 and 5 against *A. **veronii*** and was 0,3,5 and 5 against *A. **sobria*** at zero day, 4th, 8th, and 12th weeks of the experiment respectively. On the other hand the interperitoneal rout of immunization showed a high antibody titters against. A hydrophila which

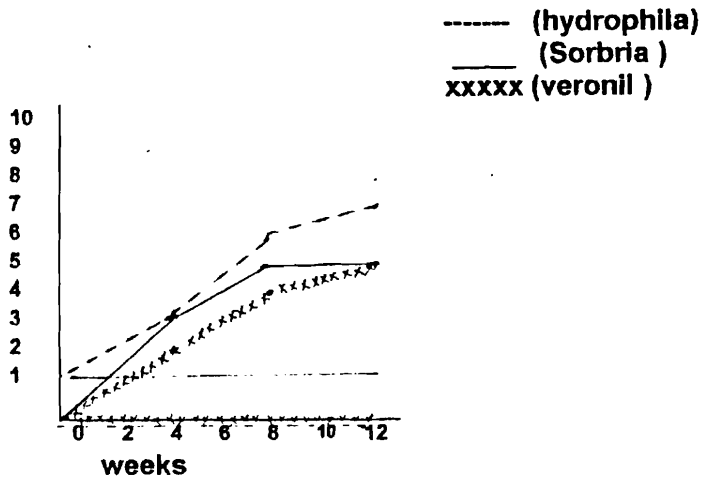

Fig. (1): Serum Aggluting anti body titres against ***A. hydrophila***, ***A. veronii*** and ***A. sobria*** of ***Oreachromis niloticus*** vaccinated orally.

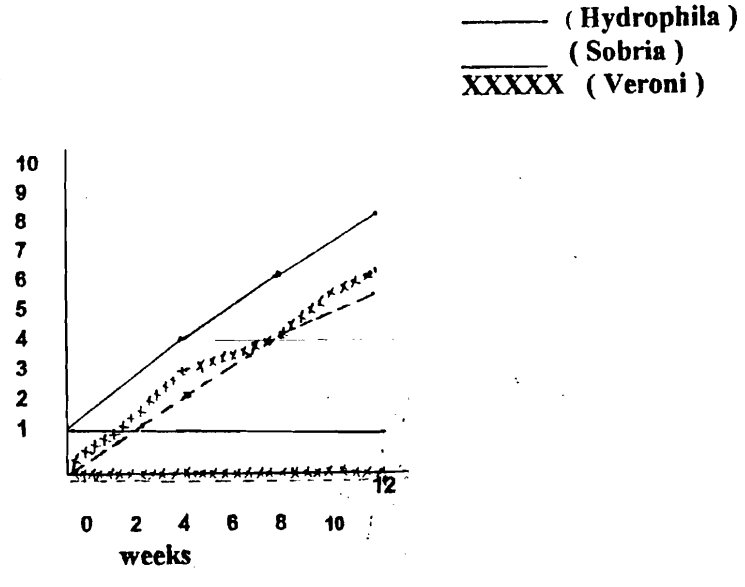

Fig. (2): Serum Agglutinating anti body titres against ***A. hydrophila***, ***A. veronii*** and ***A. sobria*** of ***Oreachromis niloticus*** vaccinated intraperitoneally.

was 1,4,6 and 8 and against ***A. veronii*** was 0,3,4 and 6 and against ***A. sobria*** it was 0,2,4 and 5 at zero day, 4th, 8th, and 12th week of experiment respectively.

b) In the control group: the agglutinating antibody titters against ***A. veronii*** and ***A. sobria*** and ***A. hydrophila*** were 0,0 and 1 respectively all over the experimental period.

II- Mortality and production:

The results presented in table (1) revealed that, fish vaccinated orally or intraperitoneally had a lower mortality % and a higher relative level of protection started as a 4th week of the experiment and extended up to 12th week, after challenge with ***A. hydrophila***. On the other hand, the results presented in table (2) revealed that both routs of vaccination protected Nile tilapia against challenge with ***A. veronii*** Mortality % were decreased specially at 4th week until reached zero at 12th week, whears RLP were increased until reached 100% at the 12 week.

III- re-isolation and identification of organisms:

All dead fish after each challenge showed clinical signs of haemorr hagic Septicaemia before death, and revealed the presence of ***A. hydrophila*** and ***A. veronii*** after challenged with ***A. hydrophila*** or ***A. veronii*** respectively. The organisms were re-isolated from the head kidney of the challenged fish.

DISCUSSION

Motile members of the genus Aermonas constitute a major problem as disease producers among a wide variety of fresh water fish. Not only Aeromonas hydrophila, as it was previously believed, but also A. Sobria, ***A. veronii***, A. jandaei, A. Schubertii and even A. Caviae were able to cause a disease spectrum in different hosts including fish and human being (Gray, 1984, Amin ***et al.***, Zaki 1991 and Gado 1994).

The results presented in this study show that Nile tilapia (***Oreochromis niloticus***) can be successfully vaccinated intraperitoneally or orally against motile Aeromonas Septicemia infection. A detectable lower mortality was

Table (1): Relative level of protection (RLP) and mortality (M %) of ***Oreochromis niloticus*** vaccinated orally or intraperitoneally (i/p) and challenged with A. hydrophila.

Groups	Route of vacc.	Zeroday		4th week		8th week		12th week	
		M %	RLP	M %	RLP	M %	RLP	M %	RLP
1	Fish orally vacc.	100	0.0	40	60	20	80	0.0	100
2	Fish ip vacc.	100	0.0	20	80	0.0	100	0.0	100
3	Control fish	100	0.0	100	0.0	100	0.0	100	0.0

Table (2): Relative level of protection (RLP) and mortality % (M %) of ***Oreochromis niloticus*** vaccinated orally or intraperitoneally (I/P) and challenged with A- veronii.

Groups	Route of vacc.	Zeroday		4th week		8th week		12th week	
		M %	RLP	M %	RLP	M %	RLP	M %	RLP
1	orally	80	0	60	25	20	75	0.0	100
2	I/P	80	0	40	50	0.0	100	0.0	100
3	Control fish	80	0	80	0	80	0	60	0

achieved in both vaccinated groups after challenge with a hot strain on ***A. hydrophila*** or ***A. veronii*** at the 4th week until reached zero at the 12th of the experiment. The relative level of production increased slower in orally vaccinated group than the interapretoneally vaccinated group.

These results generally agree with those reported by Cudmundsdottir and Cudmundsdottir (1997). They studied protection in experimental challenges with a typical frunculosis in Atlantic salmon vaccinated with an autogenous ASO bacterin (Iceland Biojec. OO), a commercial frunculosis vaccine (Biojiec. 1500), or a mixture of both vaccines was compared. Their results showed that both vaccines gave protection against an injection challenge with ASA.

However, better protection was obtained with the IBOO (homologous) vaccine. Fish vaccinated with Biojec 1500 or with both vaccines were equally well protected against ASS in a cohabitation challenge. On the other hand, no protection against classical frunculosis was achieved in fish vaccinated with IBOO alone.

There are few studies concerning the success of laboratory immunization with live attenuated bacteria, and achieved a good protection against challenge with hot heterologous bacteria, such a recorded by Oliver ***et al*** (1985), who found that vaccination with live virulent A. salmonicida cells, seem to be highly immunogenic against challenge with heterologous bacteria. Also a considerable level of production was achieved in O. niloticus vaccinated with live a virulent A. Caviae sero group 0.11 when challenged orally or intramuscularly with the virulent heterologous ***A. hydrophila*** sero group 0.11 at 5th week post vaccination (Zaki 1995).

In the present study, we used two strains of motile Aermonads (***A. hydrophila*** and A. sobria) belonging to serogroup 0.11 in the preparation of polyvalent vaccine. It is known that such serogroup exhibited certain cell surface associated phenotypic characteristics as autoaggregation in broth culture, resistance to bactericidal activity of normal serum and highly virulent strains for salmonid (Mittal ***et al***, 1980).

Later, many studies focused on the structure of surface layer (S-layer) of the highly virulent of different motile Aeromonad belonging to serogroup 0.11 and

they found that S. layer had a tetragonal morphology resemble A. layer of A salmonicida with unit cell dimension of approximately 12 mm, it contain a single polypeptide of approximate molecular weight of 50.000-52.000 Dalton, and polysaccharide chains of the lipopoly saccharide (0-antigen) are highly immunogenic. (Dooly ***et al***, 1986. Dooly and Trust 1988, Kokka and Janda 1990).

Another recent study reported by Ford and Thune (1992) found that channel catfish immunized with crude, acid – extracted S. layer protein of motile Aeromonads emulsified in Freund's incomplete adjuvant were protected against experimental challenge with the homologous virulent strain.

All motile Aeromonad used for a polyvalent cavvine were able to induce specific antibodies that increased in the serum of ***Oreochromis niloticus*** from the 4^{th} to 12 week either vaccinated orally or intraperitoneally.

The specific antibodies were mainly directed against A. layer protein of A. Salmonicida which resembles S. layer of motile Aeromonads (Gudmundsdottir ***et al***, 1997). Many authors tried to correlate between humoral antibody titters and protection as Ruangpan ***et al***, (1986), Baba ***et al***, (1988) Soliman ***et al***, (1989), Badran ***et al***, (1993) Zaki, (1995).

However, nonspecific defense mechanisms as well as cellular immune response may play some role in the protection obtained in the vaccinated fish.

In conclusion, the results obtained from the vaccination study to control motile Aeromonad infection in fresh water fish are promising Due to the antigenic diversity of motile Aeromonad presents a major problem in vaccine development, so that polyvalent vaccine might have to contain antigens representing most of the strains that fish encounter.

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