

8. METHODS OF VACCINE PREPARATION AND TECHNIQUES OF FISH IMMUNIZATION

Aim:

To develop vaccine (formalin killed whole-cell antigen) of pathogenic strain (*Aeromonas hydrophila*) and the techniques of fish immunization.

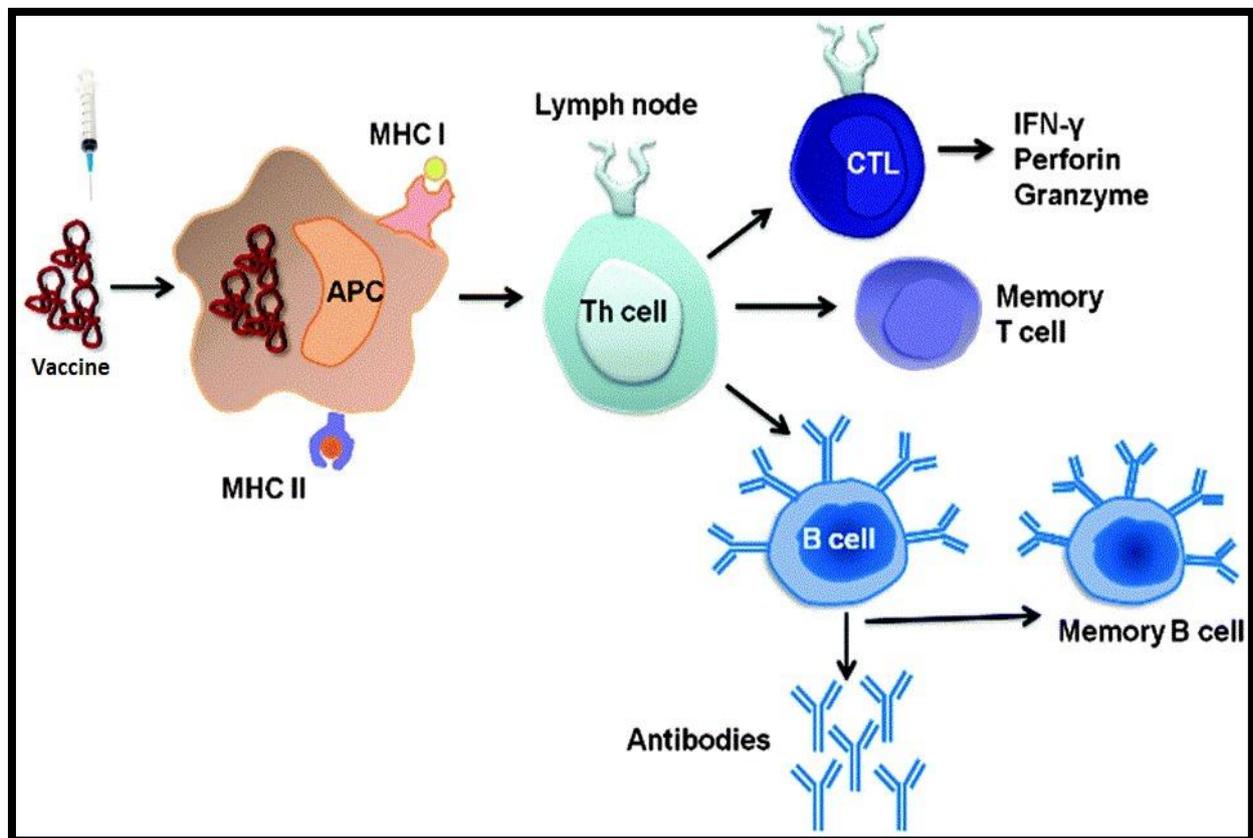
Introduction:

A vaccine is a biological preparation that improves immunity against the particular disease. Vaccines contain an agent that identified a disease causing micro-organisms & is often made from weakened or killed forms of the microbes, its toxins or one of its surface proteins. It stimulates the body's immune system to recognize the agent as foreign, destroy it & remember it, so that the immune system can more easily recognize & destroy any of these micro-organisms that it later encounters.

Characteristic of ideal vaccines:

- ❖ Should not be toxic or pathogenic i.e. it should be safe.
- ❖ Should have very low level of side effects in normal individuals.
- ❖ Should not cause problems in individuals with impaired immune system.
- ❖ Should not spread either within the vaccinated individual or to other individuals.
- ❖ Should not contaminate the individuals .
- ❖ Should be effective in producing long lasting humoral & cellular immunities.
- ❖ The techniques of vaccination should be simple.
- ❖ It should be cheap.

Mechanism of vaccine action:



Materials required:

1. Live fish
2. Tryptic soy broth (TSB)
3. Tryptic soy agar (TSA)
4. Pathogenic strain (*Aeromonas hydrophila*)
5. Petri plate
6. Test tube
7. Inoculation loop
8. Spreader
9. Incubator
10. Cotton
11. Cooling centrifuge machine
12. Centrifuge tube
13. Laminar flow cabinet
14. Bunsen burner
15. Phosphate buffer saline (PBS)
16. Refrigerator
17. Adjuvant (IFA)
18. Syringe
19. NSS

Procedure:

The cell suspension of *A. hydrophila* strain was prepared, following the method:

- i) These strains, maintained on tryptic soy agar (TSA) slants, were streaked on to fresh TSA plates and incubated at 30°C for 24 h to get young discrete colonies.
- ii) Two young colonies were aseptically picked, transferred to 10 ml of tryptic soy broth (TSB) and incubated at 30°C for 24 h.
- iii) This 24 h old culture was then transferred to 300 ml TSB and re-incubated at 30°C for 24 h.
- iv) The cells were harvested by centrifugation at 7500 rpm for 30 min at 25°C in a cooling centrifuge.
- v) The cell pellets were washed thrice by centrifugation with sterile phosphate buffer saline (PBS, pH 7.2) and finally re-suspended in 10 ml of sterile PBS and kept at 4 °C.
- vi) The number of cells/ml of suspension was determined by spread plating on TSA after incubation at 30°C for 24 h.

Administration/ immunization methods:

Three main vaccines administrating methods

- I. Injectable vaccines
- II. Immersion vaccines
- III. Oral vaccination

Injectable vaccines:

- ❖ The vast majority of immunized fish are vaccinated by injection into the peritoneal cavity.
- ❖ Vaccine is injected in the abdominal area of each anaesthetised fish (>50g). Now also micro vaccines (0.05ml).
- ❖ The needle is inserted into the peritoneal cavity at a 45° angle to a depth of approximately 0.5 cm.
- ❖ Vaccines can also delivered by intramuscular injection.
- ❖ Intraperitoneal delivery is good for healthy fish with minimum stress & protect the fish throughout the lifetime.
- ❖ Modern vaccines will protect against 5 or more different diseases from a single shot.



Immersion vaccines:

Two application methods: dip and bath

- **Dip vaccination:** More widely used. Rapid vaccination of large numbers of fish (up to 100kg of fish / litre of vaccine solution). Small fish (1-5 gm) immersed for very short duration (30 seconds) in a highly concentrated vaccine solution (1 part vaccine to 9 parts water).
- **Bath vaccination:** Larger fish are exposed for a longer period, usually one to several hours, in a lower concentration of vaccine.



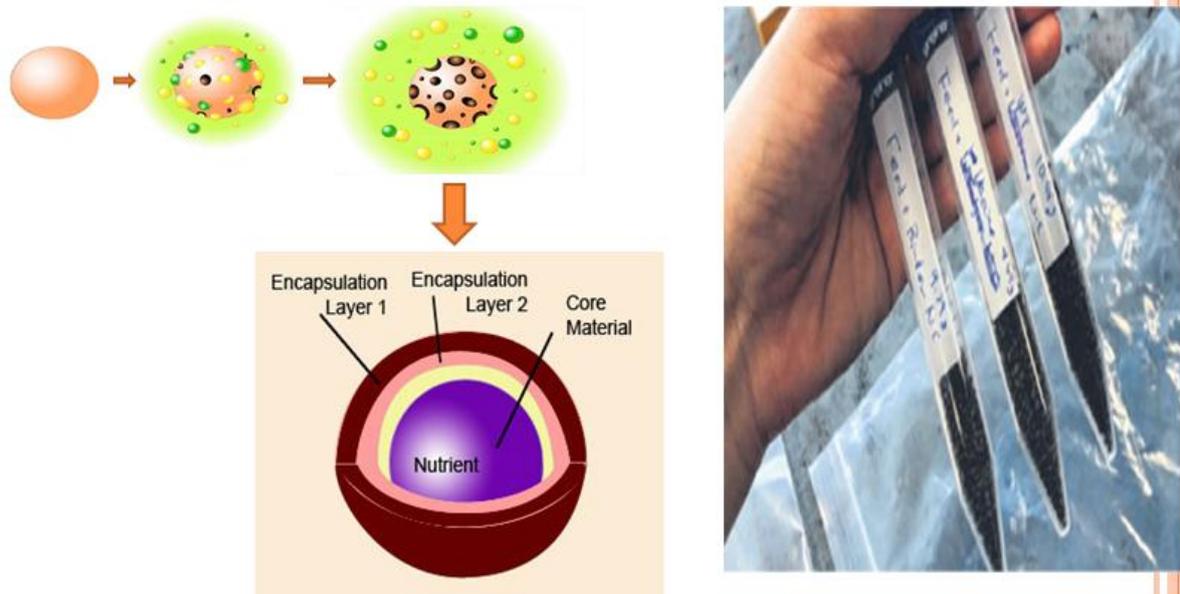
Dip vaccination



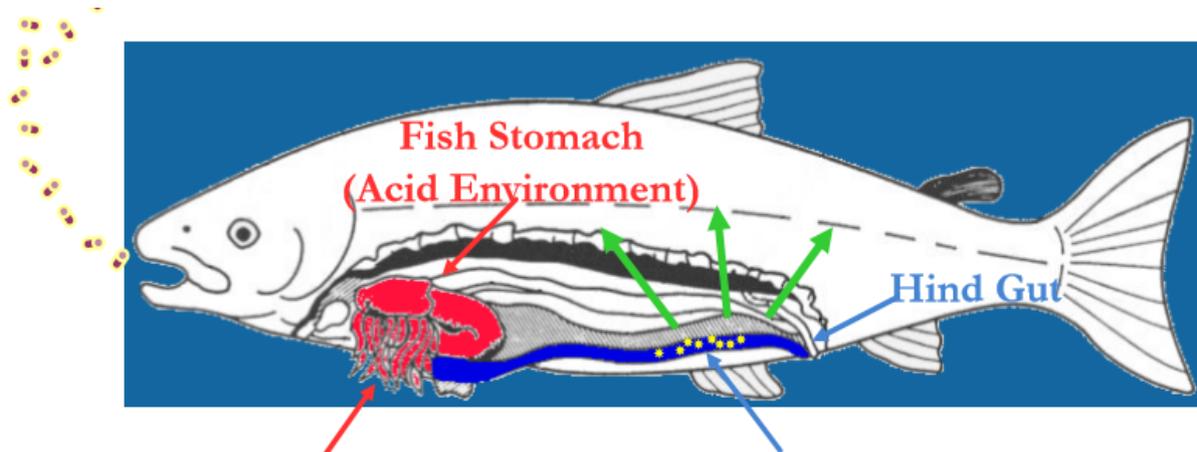
Bath vaccination

Oral vaccination:

- In oral vaccination, the vaccine is either mixed with the feed, coated on top of the feed (top dressed) or bio-encapsulate.
- Bio-encapsulation is used where fish or shrimp fry are to be vaccinated. In this case, live feed such as artemia nauplii, copepods or rotifers are incubated in a vaccine suspension and then fed to the fry.



When vaccines are used as top dressing in feed, a coating agent is usually applied, either to prevent leaching of the antigen from the pellets or to prevent breakdown of the antigen in the acidic environment of the stomach.



1. In the acid environment of the fish stomach, the feed pellets are digested. The Antigen themselves are protected by the APV and pass through intact.
2. Antigens are delivered to the area of the hind gut where they are absorbed and activate an effective immune response in the fish.

Observation:

Groups	Mortality effect at immunized/ vaccinated periods		Mortality effect at <i>A. hydrophila</i> challenged periods.	
	Number	Clinical signs	Number	Clinical signs
Injectable vaccinated group				
Immersion vaccinated group				
Oral vaccinated group				
NSS injected group (Positive control)				
Control fish				

Others observation: