



## **PATHOLOGICAL PROCESSES AND DISEASE DEVELOPMENT**

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Diseases are among the greatest deterrents to the sustained production in aquaculture. White spot diseases (WSD) in cultured shrimp and epizootic ulcerative syndrome (EUS) in fishes have amply demonstrated the serious impact of disease in aquaculture. Aquaculture medicine, which broadly encompasses prevention and management of diseases in cultured aquatic organisms, becomes a vital requirement for a sustained industry.

The four K's essential for scientific aquaculture health management is knowledge about the disease process, knowledge about the pathogen, knowledge about the host and the knowledge about the environment. Disease development process is often complicated and involves host-pathogen-environment interactions. With respect to the pathogen, the knowledge as to how it attaches and enters the host, derives nourishment, reproduces, gets transmitted, overcomes host defense barriers, etc are very important. Understanding the susceptibility aspects of a host to a given pathogen is important. It will depend on host species, its age, size, immunocompetence and stress response. Knowledge about the temporal and spatial aspects of environment on disease, how they stress the host and favour the pathogen are vital

### **Process of Disease Development**

There exist a delicate balance between the host, pathogen and the environment. When this delicate balance gets upset, disease can result. Aquaculture environments can stress the host, favour the pathogen and result in disease development. Understanding the pathogenicity mechanisms of the pathogens, disease resistance mechanism of the host and the role of the environment is essential to appreciate the process of disease development. To gain insight into the process of disease development, it is very essential to understand the role of various pathogens, the adaptive modifications they have, the functional importance of the target tissue, interaction between pathogen and host at the target tissue level, pathogenicity mechanisms of pathogens, etc. A pathogen can cause a clinical disease only when it can establish on or in the host, proliferate, overcome the non-specific and/or specific defense barriers of the host, produce the pathogenic factors, cause cellular and tissue damage, produce significant pathological changes, impair the function of the target tissue and cause mortality. This process is very complicated. Stress and environmental factors can accelerate this process. The sequence of disease development will to a large extent depend on the nature of the pathogen (parasite, bacteria, fungi, virus), environmental factors, size of the host, pathogen load or intensity per unit area or unit weight of the host.

### **Role of Stress in Disease Development**

The role of stress in predisposing the fish/shrimp to infections is widely recognised. It is now well known that many of the routine aquaculture practices and pollutants can stress the fish and predispose them to disease. Stress is a non-specific

response and it involves series of changes in the animal in trying to adapt to the changed situation. Stress is produced by environmental or other factors which extends the adaptive responses of the animal beyond the normal range or which disturbs the normal functions to such an extent that the chances of survival are significantly reduced. The series of changes termed "stress response" tries to help the animal restore the normal homeostasis. This process has both advantages and disadvantages. During stress, hypothalamus- pituitary-interrenal axis (HPI axis) gets stimulated and increases the output of stress hormones called corticosteroids. These stress hormones help to mobilize additional energy during the response to regain the internal homeostasis. The concentration of cortico-steroids comes down to basal level after the withdrawal of the stimuli. On the other hand, stress hormones are basically immuno-suppressive in nature. This immunosuppressive properties of the stress specific hormone can significantly lower the efficiency of both non-specific and specific immune system of fish and can render the animal more susceptible to disease.

It is well known that common husbandry practices like handling, netting, transportation and the normal features of an intensive culture system like suspended solids, low oxygen, high organic matter, overcrowding, high ammonia, etc. can elevate the level of corticosteroids in the blood. During these phases, the fish may easily get infected if there are sufficient number of pathogens in the water. Similarly, many of the pollutants at very low levels can stress the fish and elevate the level of corticosteroids in the blood and make the fish relatively more susceptible to infection. Many of the stressors encountered in intensive culture systems are of chronic nature and can keep the level of corticosteroids above basal levels for longer duration. Therefore, minimizing stress factors is the key to successful health management.

### **Pathological Processes**

Pathology is the basis of medicine. It tells us what is happening at the tissue level. Only through pathology, host-pathogen interactions can be best understood and appreciated at structural, functional, microscopical and ultrastructural levels. Pathology in simple is the outcome of three basic processes:

- (a) cellular responses to pathogen induced injury
- (b) inflammatory response exhibited by the host
- (c) pathogenicity mechanisms of the pathogen

The interaction of these three processes in the target tissue of the host produce series of pathological changes. Functional failure of the target tissue leads to clinical manifestation of the disease followed by morbidity and mortality. Pathology will thus provide an insight into many of these processes and help to evolve scientific health management package.

### **Cellular Responses to Injury**

The cellular environment is constantly changing and as a consequence, cells have to make continuous adjustments to accommodate these changes. The cells have a great capacity for adaptation to their environment and are able to respond to changes in the internal and external environment by alterations in both their structure and function. For example, adipose tissue cells respond to prolonged excessive food intake by increasing their synthesis of storable fat (increase in the size of adipocytes = hypertrophy). Skeletal

muscle fibers increase in size in response to increased work load as seen in athletes. These are examples of cellular responses (physiological hypertrophy) to physiological stimuli.

Certain changes lie outside an acceptable physiological range. Such adverse changes may be termed as pathological stimuli. Cells may respond to pathological stimuli by extending their normal physiological adaptive processes. These adaptive processes include:

(a) Increased cellular activity

- i) increase in cell size (hypertrophy)
- ii) increase in cell number (hyperplasia)
- iii) enzyme or metabolic induction

(b) Decreased cellular activity

- i) reduced cell size (cell atrophy)
- ii) reduced number of cells (tissue or organ atrophy)
- iii) diminished cellular metabolic function

(c) Change in morphology and function of mature cell type

- i) modification in morphology and function to a cell type more suited to the changed environment (metaplasia)

### **Failure of Cellular Adaptation**

Cells, which are intrinsically unable to adapt, or which have reached their limit of adaptability, begin to show structural changes, which indicate their failure to withstand the changed environment. These cells show cytoplasmic and nuclear changes as a manifestation of organelle failure (cloudy swelling, hydropic degeneration, fatty change and cell necrosis). Both hydropic and fatty degeneration are reversible if the deleterious stimulus is short lived. If adverse conditions persist however, or if the initial pathological stimulus was severe, then these changes continue and progress into a sequence of events leading to cell death (cell necrosis). The onset of these irreversible changes is heralded by distinct morphological changes in the cell cytoplasm and the cell nucleus.

The initial responses of cells to injury are manifested at a subcellular level by morphological changes in the various cytoplasmic organelles. Membrane enzyme systems such as those constantly engaged in maintaining ionic gradients and membrane transport are particularly vulnerable to pathological influences. One of the earliest consequences is the loss of efficiency of the sodium pump permitting ingress of sodium ions and water, resulting in swelling of the cell. This change is potentially reversible. If the cell sustains irreversible damage, mitochondria become more swollen and disruption of oxidative phosphorylation deprives the cell of aerobic metabolism. The resulting collapse of many intracellular homeostatic mechanisms leads to progressive disintegration of nuclear and cytoplasmic organelles and release of lysosomal enzymes, leading to autodigestion of the cells.

### **Acute Inflammation**

Living tissues sustain injury from a wide variety of physical, chemical, microbial or immunological causes, and the response in dealing with resulting tissue damage or destruction is known as inflammation. Whatever the cause or type of tissue involved, the initial series of processes which classically ensues is described as acute inflammation and

is directed towards neutralizing the injurious agents and to restoration of tissue to useful function. Inflammation is a non-specific, protective, vascular response. Damage of mast cells during an injury releases vaso active amines which have an influence over the microcirculation of the area. The characteristic feature of acute inflammation is the formation of an inflammatory exudate which has three principal constituents: fibrin, serum and leucocytes, predominantly neutrophils. The formation of the inflammatory exudate involves three vascular processes and functional blood supply is an essential requirement for inflammation to proceed:

- i) dilation of local blood vessels leading to engorgement with blood (hyperaemia)
- ii) increased capillary permeability permitting plasma proteins to pass into the tissue
- iii) migration of leucocytes from blood vessels into the area of injured tissue

The outcome of acute inflammation depends on three major factors: the degree of tissue injury, the nature of the injurious agent and the type of tissue involved. When tissue damage is minimal, the exudate is reabsorbed into nearby vessels leaving no subsequent evidence of injury, this process is known as resolution.

More frequently, the exudate undergoes a process called organisation and repair in which dead tissue is removed by phagocytosis and the defect is filled by a highly vascular connective tissue called granulation tissue, this then progressively undergoes fibrous repair with the formation of a dense fibrous scar at the site of the original tissue destruction. Depending on the type of tissue damage, there may be also some degree of regeneration of original tissue, which depends on the ability of cells of mature tissue to undergo division.

When tissue damage is caused by certain types of bacteria, then large number of dead and dying neutrophils accumulate with fibrin and fluid of the acute inflammatory exudate to form a localised collection of pus known as an acute abscess.

In some circumstances, an injurious agent persists over a prolonged period causing continuing tissue destruction whilst at the same time the body is attempting to deal with the previous tissue damage by the process of acute inflammation, organization and repair, in such a case the damaged area may exhibit tissue necrosis, acute inflammatory exudate, granulation tissue and fibrous scar tissue concurrently. This process is known as chronic inflammation.

### **Chronic Inflammation**

In most cases the injurious agent is destroyed or neutralised in the earlier stages of the acute inflammatory reaction, and the rest of the changes follow in sequence. Sometimes, however, the damaging stimulus persists despite the tissue responses directed at destroying or neutralising it, and further episodes of tissue destruction may result. In such circumstances the changes of tissue damage, acute inflammation, granulation tissue formation and attempts at fibrous repair may all proceed concurrently instead of sequentially as they do when the injurious agent is eliminated early in the sequence. This phenomenon is known as chronic inflammation and it most commonly follows previous acute inflammation where that process had failed to eradicate the damaging stimulus.

Chronic inflammation also follows many acute abscesses; bacteria frequently survive and proliferate in the pus at the centre of the abscess cavity and are not easily accessible to endogenous or therapeutic bactericidal substances. The wall of the chronic abscess is composed of fibrous granulation tissue, with an inner zone of acute inflammatory exudate bordering the pus filled cavity.

Less commonly, chronic inflammation occurs virtually *de novo* in response to specific damaging agents which are resistant to destruction by neutrophils during the acute inflammation or which fail to excite a strong acute reaction. When the damaging agent is not destroyed by the neutrophils, the initial neutrophil response is usually sparse and short lived, and is quickly followed by a macrophage response which persists and dominates the histological picture. This local macrophage accumulation produces a discrete lesion called a granuloma, and the chronic inflammation characterized by this type of response is known as chronic granulomatous diseases. The outcome of the chronic inflammation thus depends on whether local and systemic factors favour the injurious agent or alternatively, the attempts at healing and fibrous repair. Chronic inflammations are often of considerable duration, in which immunological mechanisms play an important role.

In summary, chronic inflammation is marked by continuing tissue damage, acute inflammatory exudation, organisation and fibrous repair occurring concurrently rather than sequentially.

#### **Pathogenicity Mechanisms**

Diseases of aquatic animals are caused by parasites, fungi, bacteria, viruses and non-infectious agents. The mechanisms ectoparasites use to establish on the host and derive nourishment, induce necrotic changes at the cellular and tissue level. Adaptive and inflammatory response exhibited by the host adds to the pathological picture in the case of ectoparasitic diseases. Necrosis and proliferative response are common pathological features associated with ectoparasitic protozoans. Ectoparasitic metazoans (worms and crustacean parasites) cause attachment and feeding injuries resulting in skin and gill necrosis and tissue proliferation in the host. In the case of endoparasites, mechanisms of penetration and migration, mode of deriving nourishment, mode of reproduction and route of exit from the host, will all contribute to the pathology. Tissue necrosis, enteritis, fibrous encapsulation, nodule formation, xenomas, etc are some of the common endoparasite associated pathological changes. Some of the endoparasites like histozoic and celozoic sporozoans despite causing massive tissue destruction do not elicit any inflammatory response from the host.

In the case of bacterial diseases, enzymes, toxins, haemolysins and other histolytic factors produced by the proliferating bacteria induce severe pathological changes. Surface ulcerative bacterial diseases (vibriosis, columnaris) show necrotic and proliferative changes at the surface leading to development of ulcers. Acute systemic bacterial diseases (bacterial haemorrhagic septicaemia, furunculosis, enteric red mouth) produce areas of focal to general necrosis in affected organs. Chronic granulomatous type of bacterial diseases (bacterial kidney disease, fish tuberculosis) produce granulomas in various organs and tissues. In shrimp, bacterial infections like oral, cuticular, enteric and systemic vibriosis produce massive necrotic changes in the affected organs. Characteristic

haemocyte led inflammatory response in the form of melanised haemocytic nodules are a common feature of shrimp vibriosis.

In the case of fungal diseases, the histolytic properties of the invading fungal hyphae induce necrotic changes in the concerned target tissue. In dermal mycosis, the invading fungal hyphae brings about severe necrotic changes in the integument. Some of the fungal pathogens (*Saprolegnia*) are able to invade the host tissue and cause destruction without stimulating any inflammatory response. Many of the systemic fungal pathogens (*Ichthyophonus*, *Exophiala*) produce characteristic mycotic granulomas. *Aphanomyces invadans*, the necessary cause of EUS, produces mycotic granulomas in all the affected target tissues. In shrimp, fungal infections like *Lagenidium*, produce necrotic tissue pathology without any significant inflammatory response, while *Fusarium* infection induces typical granulomatous response.

Viruses are intracellular obligatory pathogens entirely dependent on host cell machinery for their replication. Viral replication induces areas of focal to general necrosis of cells in the target tissue. Apart from complete destruction of cells, viral infection can lead to enlargement of cells (giant or syncytial cells) or partial to complete loss of function. Viral infections produce intracytoplasmic or intranuclear viral inclusion bodies, which are of diagnostic significance. For example, the WSSV produces basophilic intranuclear inclusion bodies in the cells of ectodermal and mesodermal origin. Of significance is the fact that viral infections do not normally produce any inflammatory response.

#### **What is not Pathology ?**

While using pathology for diagnostic purpose or understanding disease process, it is very important to appreciate what is true pathology and what is not. Physiological changes in the cell structure and function to physiological stimuli are not pathological changes. It is very essential to differentiate post-mortem changes from true pathological changes. In this context, it is necessary to emphasise the need for using only live or moribund aquatic samples for all histopathological diagnostic purposes. Artifacts resulting from poor sample fixation, processing, sectioning and staining should be clearly differentiated from true pathology. It is very common to see artifacts being read and published as pathology. This is largely due to the lack of training in histopathology.

#### **Applications of Pathology in Aquaculture Medicine**

Pathology is in importance, at least first among equals, being the basis of medicine on which health management is built. An accurate appreciation of what is happening in the tissues will form the basis of health management and aquaculture medicine. Histopathology provides the simplest and easiest means of learning about interactions going on between the host and the pathogen at the cellular level. The advantage of histopathology is its being a non-specific diagnostic tool. It can be used to diagnose parasitic, bacterial, fungal and viral diseases of aquatic organisms. Histopathological inferences can be put to various uses such as:

- for understanding the disease process
- for diagnosis of parasitic, bacterial, fungal and viral diseases
- for screening, certification and quarantine purposes

- for epidemiological analysis
- for understanding multiplication and transmission issues of pathogens
- for assessing the accessibility of a pathogen for chemotherapy
- for assessing the inflammatory response of the host to the pathogen
- for assessing whether immuno-prophylactic strategies can be used for a pathogen
- for formulating scientific and comprehensive health management strategies.

Through rational interpretation of histopathological findings, it is possible to arrive at conclusions on the pathogenicity mechanisms of pathogens, functional status of target organs, severity of a disease, cause of morbidity and mortality. However, histopathology being a non-specific diagnostic tool suffers from certain limitations, but the advantages of using histopathology for aquatic animal health diagnostics and management overweighs its limitations.

The pathological picture one sees in a tissue is therefore the combined effect of pathogenicity mechanisms of pathogens, cellular response to injury and the host inflammatory response. Understanding the pathology helps to appreciate the severity of the disease, the likely clinical signs, the possible approaches to management, etc. In summary, pathology and the disease process form the foundation for scientific health management.