Winter School on

'RECENT ADVANCES IN
DIAGNOSIS AND
MANAGEMENT OF DISEASES
IN MARICULTURE'

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The importance of aquaculture to food security is well known. Aquaculture development therefore, has assumed significant momentum in several parts of the world. Diseases are among the greatest deterrents to the sustained production in aquaculture. Diseases like Epizootic Ulcerative Syndrome (EUS) in fishes and White Spot Syndrome (WSS) in shrimps have amply demonstrated the devastating effect of disease outbreaks on aquaculture productivity and profitability. Various diagnostic and health management tools are being used to devise disease control strategies. It is becoming increasingly difficult to manage pathogens that have become endemic. Epidemiological approaches which have been very successful in human and veterinary medicine are now being increasingly recognised as important weapons that can be used in aquaculture to formulate disease control programmes.

Epidemiology

Epidemiology is the study of disease in population in its natural setting. Epidemiology is population medicine. For the epidemiologist, the population is the patient. All epidemiological studies are field based. In aquatic epidemiology, aquaculture ponds serve as the laboratory. This can be a great advantage over laboratory studies, which cannot be easily extrapolated to field conditions.

Cause from an epidemiological perspective is interpreted in quite a wide sense. This is somewhat different to the more traditional view of the role of cause being restricted to etiological agents. An epidemiological definition of a cause of a disease is "an event, condition or characteristic that plays an essential role in producing an occurrence of the disease". Epidemiologists avoid defining the word cause for any disease outbreak, but prefer to use words such as determinants, exposures and risk factors. Alternatively, they will categorise causes as direct or indirect; necessary or sufficient; and single or multiple, rather than defining primary etiology.

Concept of Cause

For most diseases, there is strong evidence that disease outbreaks occur only when a number of causal factors combine. The multifactorial nature of disease causation can be represented using the concepts of necessary cause, component cause and sufficient cause. Each combination of various causal factors (component causes) which together cause a disease collectively as a "sufficient cause" for that disease. It is important to recognise that, under different circumstances, different combinations of "component causes" may constitute sufficient cause for a disease. Disease outbreaks will occur only if there is a sufficient cause for that disease. Moreover, all sufficient causes for a particular disease have in common at least one component cause, known as "necessary cause". This necessary cause must always be present for that disease to occur. More presence of necessary cause alone will not always cause the disease.
Under such a definition, the presence of say WSSV (necessary cause) alone in a pond of shrimp may not of itself be a sufficient cause for WSS to occur. It may require a stress trigger to cause an outbreak. Under this concept, the WSSV is a necessary cause (no disease would occur if WSSV was not present) but not a sufficient cause of the particular syndrome, whereas the stress is neither necessary nor sufficient but can be a component of a sufficient cause. In fact, for any particular expression of a particular disease, there may be a range of possible sufficient cause complexes.

Similarly, presence of *Aphanomyces invadans* (necessary cause) alone is not sufficient for EUS to occur. For EUS to occur, combinations of causal factors must ultimately lead to exposure of dermis, attachment to it by *Aphanomyces invadans* propagules (the only currently recognised necessary cause) and subsequent invasion by the fungus of dermis and muscle. The resulting mycotic granulomatous dermatitis and myostitis are, by case definition, EUS. Interventions aimed at eliminating any one of the component causes can prevent occurrence of EUS despite the presence of the necessary cause.

On the other hand, the aetiology of a disease outbreak may be well defined, however, the cause of the same outbreak may be confusing. For example, two ponds may test PCR+ for WSSV but only one pond may experience a disease outbreak. In this instance, WSSV (necessary cause) must be present for the outbreak to occur but the presence of WSSV in the pond doesn’t necessarily lead to an outbreak. Likewise in two PCR+ ponds, one may experience high mortalities while the other low mortalities. Therefore, there are other risk factors (component causes) which would determine whether the disease is expressed and if it is expressed, the severity of the outbreak.

**Risk Factors**

Risk factors are those characteristics on the basis of population based evidence which are associated with increased risk of disease. Protective factors are those which are associated with decreased risk of disease based on a population evidence. Risk factors may be either causal or non-causal. Epidemiological studies have the objective of identifying these risk factors, quantifying their effect on outcome, and formulating intervention strategies on a pond, farm, region or country level. The challenge for the epidemiologist is to help identify some of the more important components of sufficient causes for a particular disease with the view to devise cost-effective intervention strategies at critical points to either prevent disease expression or reduce the production effects.

Epidemiologists use three rules to reject a hypothesised cause (risk factor). The first is association. If a risk factor does not occur more frequently in diseased animals, then it is rejected as a cause. The second is time order. If the risk factor does not precede the outcome, it is rejected. The third criterion used is common sense or coherence. Risk factors are often rejected if they do not make any biological sense.

**Outcomes and Associations**

Epidemiological studies define certain outcomes and use them for separating the population into groups for comparison. Frequently used outcomes in epidemiology are mortality and disease outbreaks. For example, shrimp ponds may be divided into successful ponds and failure ponds based on disease outbreaks, yield, weight at harvest, length of production cycle, etc. Using epidemiological and statistical tools, it is possible
to identify variables (factors) associated with defined outcomes. Variables statistically associated with successful outcomes are regarded as protective factors. Variables statistically associated with failure outcomes are regarded as risk factors. The risk/protective factors identified based on a population are mere associations with the outcome and should not be regarded as either cause of the outcome or solution to the outcome.

Disease as Outcomes
In aquatic epidemiological studies it is very common to use disease as outcomes. Several aquatic epidemiological studies are being conducted to identify factors associated with outbreaks of WSS and EUS. Whenever, disease is used as an outcome in epidemiological study, it is very important to have a case definition for the disease outcome to maintain consistency. A case definition is a set of standard criteria for deciding whether an individual unit of interest (fish/pond) has a particular disease. If different criteria are used, errors are likely to be introduced. For example for WSS, case definition can include clinical white spots, pink coloration, increasing mortalities, emergency harvest, PCR positivity and intranuclear inclusions of WSSV. Similarly for EUS, surface ulcers, mycotic granulomas in histology, isolation of *Aphanomyces invadans*, seasonality and species range can be included in the case definition.

Quantitative Epidemiological Studies
Quantitative epidemiological studies can be broadly grouped into observational, intervention and theoretical studies.

Observational Studies
In observational studies, nature is allowed to take its course while the differences or changes in characteristics of the population are studied without intervention from the investigator. Observational studies can be descriptive, longitudinal, cross-sectional or case-control. Descriptive studies describe the distribution and frequency of a disease in a population in terms of animal, place and time. The described patterns may lead to an hypothesis (for example, EUS occurs normally during cooler months of the year). Longitudinal or cohort studies follow study units through time, observe and record the course of natural events, record frequency of disease of interest, look for differences (factors) between groups with and without the disease. Cross-sectional study gives a snapshot in time, prevalence of disease is measured and compared among those with and without the risk factor of interest (ex: PCR positivity of PL). Case-control study selects units with the disease of interest as cases and units without the disease as controls, frequencies of suspected risk factors are then measured for the two groups and compared.

Intervention and Theoretical Studies
Intervention studies are epidemiological experiments imposed at the population level. The purpose is to evaluate some preventive or treatment or management strategy. This population based approach can be effectively used to screen and evaluate some of the commonly used preventive and therapeutic chemicals and formulations (for example, Vitamin C, immunostimulants, etc. in shrimp culture). Theoretical approaches use mathematical modelling using computers to answer "what-if" type of questions.
Steps in Designing and Execution of an Aquatic Epidemiological Study

The first thing is to have clear questions or objectives for the study (for example identification of risk factors for WSS outbreaks). This is followed by identification of the target population (shrimp farmers) and the study unit (pond). At this stage, it is very important to involve and understand the population. Most epidemiological studies of this type will be observational studies. The next step is deciding on the study design. This will depend on the physical and financial resources. Once the study is designed, data collection needs to be planned through questionnaires, measurements, sampling, laboratory tests, etc. The data gathered has to be analysed using epidemiological models to identify risk factors associated with defined outcomes (WSS outbreak).

Sampling is a crucial issue in epidemiological studies. The sample should be random and should represent the target population. The results obtained from the sample should have to be generalised to the whole population. In order to achieve this, the sample size should be such that the results could be generalised with 95% confidence to the whole population and should have the power to show the statistical differences between groups. Problems of bias and how to avoid it are of central importance to the validity of all epidemiological studies. Bias occurs when observations do not reflect the true situation. This is because of systematic error such as sample selection bias, sampling bias and measurement bias.

The aim of data analysis is to identify risk factors for disease (WSS) outbreaks. Risk factor is any variable having statistical association with the disease outbreak of concern. Positive association suggests increased risk of disease (risk factor) while negative association suggests decreased risk of disease (protective factor). Such identified associations are only statistical associations and not the same as the cause. The strength of the association is measured by relative risk (RR)/odds ratio (OR). The RR/OR of 1 indicates no association. > 1 indicates increased risk. < 1 indicates decreased risk.

Univariate and multivariate analyses are the methods used for data analysis. In univariate analysis, all the variables are examined individually and looked for associations with the defined outcomes. ANOVA, Chi square, Fishers exact and contingency table are some of the univariate tests. In multivariate analysis, effect of each variable when adjusted for the effects of all others is looked into. Logistic and linear regression models are some of the multivariate tests.

Relevance of Epidemiology to Aquaculture

Several pathogens have become endemic and established in natural populations. WSSV in shrimp is a good example. It is almost impossible to eliminate some of these well established endemic pathogens like WSSV which is the necessary cause of WSS. Eliminating/avoiding/ minimising any one of the component causes (risk factors) can prevent the occurrence of WSS despite the presence of WSSV. The challenge for aquatic epidemiologists is to conduct population based studies, identify component causes (risk factors) for disease outbreaks, devise cost effective intervention strategies at critical points. These could include strategies to prevent disease expression, to minimise the severity of disease or to minimise production losses. Recent findings from epidemiological studies on EUS have found out several risk factors associated with EUS outbreaks in culture ponds. By managing some of these risk factors, it has been possible
to prevent EUS outbreaks despite the presence of the necessary cause. Similarly, epidemiological evidences coming from studies on WSS have successfully identified several risk factors. Managing some of these risk factors it has been possible to minimise the impact of WSS outbreaks despite the presence of the necessary cause (WSSV). Epidemiology holds great potential for aquatic animal disease management.