



Quantitative Analysis of Biosecurity Levels in Fish Farms Based on a Scoring System. A Case Study in Sri Lanka

Pramodi Sembapperuma* and H.M.N. Padmasiri

Department of Business Economics, University of Colombo, Colombo, Sri Lanka

*Corresponding author: Pramodi Sembapperuma, Department of Business Economics, University of Colombo, Colombo, Sri Lanka, Tel: 718245018; E-mail: pramodijis2013@gmail.com

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Abstract

The importance of biosecurity levels vary, as every pathway of disease transmission is not the same. The risk based quantitative assessment, which is more insightful, compared to general qualitative assessment, and therefore, could be considered as the ideal way to identify the gaps in different biosecurity measures in detail. All the possible ways of transmitting pathogens and the relevant preventive measures in fish farms have been included in the analysis used in the study and it has been further sub divided into external and internal biosecurity. In this, relative importance of different biosecurity aspects has been taken into account and accordingly, the final score is weighted in developing the risk based weighted scoring system. The biosecurity score obtained was indicated after the completion of the questionnaire and the scores at each subcategory can be compared by the farmer or the field veterinarian. A study carried out using a sample of 91 fish farmers in Gampaha district, revealed that there is a vast variation in the scores of biosecurity level in fish farms, ranging from 63% to 65% and this implies that many biosecurity measures have not been adequately implemented in these farms and there exists more room for improvement.

Keywords: Biosecurity; Scoring system; Risk based; Field veterinarian; Biosecurity

Introduction

Performance, thus the profitability suffers greatly of the business entities that operate in ornamental fish industry whenever there is an outbreak of a disease. Although the economic losses to the individual farmer is calculated when there is an outbreak of an endemic disease, systematic quantification of social and other related impacts, for example the impact on trade and employment, chemical and drug usage and environment has never been done. In case of an outbreak of an epidemic disease, the entire production system gets disrupted and the infected has to be placed under quarantine [1]. Improper environmental factors, inadequate farm management, uncontrollable

movements in aquatic animals have been cited as the major factors that result in outbreak of disease. In controlling the aquatic diseases, the relationship between host, pathogen and the environment should be thoroughly understood [2].

This is the place where biosecurity plays a pivotal role in ornamental fish farming in preventing outbreak of disease, rather than in curing them. As a result, biosecurity is defined as all the measures taken in to consideration to prevent the introduction and spread of infectious agents within the farm in order to keep the fish healthy and also to limit the spread of pathogens in the environment [3].

Biosecurity management can be categorized into two, namely external and internal biosecurity management [4]. External biosecurity is related to the measures that prevent pathogens entering the farms while internal biosecurity is related to the measures used to minimize the pathogens within the farm [5].

Therefore, careful identification of gaps in each level of farm management practices is worthwhile. Hence, there should frequent assessment of the biosecurity management practices for compliance as there is always the risk of exotic or endemic disease spreading into the farm. Good evaluation of biosecurity system in each farm is required to address the issue.

More often checklists based on qualitative assessment indicators are used to assess the biosecurity levels in farms [6]. Recently most scientists have identified that the importance of biosecurity levels is not the same as every pathway of disease transmission is not equally efficient. Therefore, method of risk based quantitative assessment developed paving the way for more insightful assessment, compared to general qualitative assessment. Under the said system, different weights will be given for each biosecurity measure to calculate the final score. This helps in identifying the gaps in different biosecurity measures in detail. Quantitative assessment tools of biosecurity levels for pig herds and poultry flocks have been extensively used in most of the developed countries [7]. A large number of risk factors related to biosecurity measures in different livestock and poultry productions systems have been identified in scientific literature but the available scientific facts specific for biosecurity in ornamental fish farms are very few. There is no such quantitative risk assessment system available in Sri Lanka for ornamental fish farms, not to the knowledge of the researchers of this study [8].

To develop a quantitative biosecurity assessment system to assess the biosecurity status of ornamental fish farms in Sri Lanka is the major objective of this study [9].

Materials and Methods

Selection of farms

Ornamental fish farms in Gampaha district were selected for the study as it is considered as one of the districts consisting higher number of ornamental fish farms in Sri Lanka and as the district having the highest number of fish farmers that engaged in export activities [10].

As reliable data that had been recorded previously on fish farmers could not be obtained, all the ornamental fish exporters currently engaged in export activities and officially registered in animal quarantine station, department of animal production and health, Sri Lanka within the sample area were included in the study. In the absence of official registry of ornamental fish farms, farms were first

located with the help of the exporter using snow ball technique [11]. (Manager of the each export establishment assisted the researchers in identifying the fish farmers supplying fish for the exporter and the process continued until the whole area is covered). Face to face interview between the researcher and the ornamental fish farm owner, personal observations and instructions provided by the researcher were used in carrying out the questionnaire survey [12].

Development of the questionnaire

The questionnaire developed aims at describing the complete biosecurity situation at an ornamental fish farm [13]. Questions were asked on each relevant aspect of biosecurity measure considering the disease transmission routes in ornamental fish to determine whether a preventive measure is applied or to identify whether a specific situation is present or absent. The questionnaire was developed after a thorough literature survey on disease transmission in ornamental fish, based on the information obtained from the Biocheck.Ugent tool for pigs, poultry biosecurity measures and the biosecurity questionnaires available in the web sites, O.I.E and FAO [14].

A thorough literature survey was carried out using the literature published in the recent past on disease transmission in fish. All possible transmission routes were listed, for example, airborne transmission, food borne transmission, vector-borne transmission (e.g., personnel, wild birds, insects, litter, equipment, rodents or pets etc.), and environment (e.g., cleaning and disinfection the fish farm). Risk associated with each above stated situation has been identified and biosecurity measures were fixed accordingly. Information on general biosecurity procedures that are equal for every animal species (hygienic protocol before entering the farm, traffic control etc.) was obtained from the Biocheck.Ugent tool for pigs and poultry [15].

Biosecurity program can be designed for a specific disease focusing on the measures towards that disease, or it can be more generic and can be designed to reduce the risks common to different diseases. In any case, as a first step, biosecurity assessment should be carried out to establish a list of undesirable diseases and to identify the routes through which they are more likely to enter the farm, so that the most effective preventive measures can be placed, accordingly [16].

Subsequently, all the biosecurity measures that prevent the introduction of pathogen to the farm were filtered from the above mentioned information sources. Most of the biosecurity measures were related to either blocking the different entrance pathways of pathogens or the breaking of infection cycles [17]. After categorization of the scoring system into two, as external and internal biosecurity, external biosecurity was further divided into 7 subcategories and internal biosecurity into 3 subcategories.

Accordingly, the scoring system is separated into 2 main categories, external and internal biosecurity, and the questionnaire comprised of questions on different biosecurity measures. The questionnaire has been prepared with a view to extracting information on biosecurity measures in detail [18]. External biosecurity is comprised of all the measures that prevent the introduction of off farm pathogens and it is divided into 7 subcategories: Namely, purchase of new fish to the farm, feed and water supply, removal of waste water and dead fish, entrance of visitors and personnel, supply of materials, infrastructure and biological vectors, and location of the farm. Internal biosecurity includes all the measures that aim at preventing the spread of pathogens within the farm and is divided into 3 subcategories: Namely, disease management, cleaning and disinfection, and materials

and measures between compartments. Under each subcategory the number of measures included, ranged from 3 to 7 [19].

Development of biosecurity score form and validation

Biosecurity scoring system: A technical scoring system was developed taking the risk of biosecurity measures into account. Risk score of each measure was derived by taking the mean of each score given by the panel of experts and further supported by the literature survey. The prioritization and weighing of various biosecurity measures and (sub) categories have been done by ornamental fish experts, each with their own area of expertise. Veterinary practitioners and experienced ornamental fish farmers were included in the panel of experts to provide a balanced view on the importance of individual measures. The method described by Gelaude was considered in quantifying the effect of a specific biosecurity measure. Biosecurity measures were divided into external and internal biosecurity measures and external biosecurity measures comprised of seven subcategories and the internal biosecurity measures comprised of three subcategories.

It is well known that direct contact between animals (purchase of live animals, several animal species on one farm) poses a higher risk, whereas indirect contacts (e.g., transmission of pathogens by rodents, sharing of material between different farms) are less forceful in the transmission of pathogens. The above mentioned difference in disease transmission has been taken into account in the biosecurity scoring system by weighing the different preventive measures accordingly [20].

In general, the internal biosecurity scores were higher than the external biosecurity scores in studies conducted in dairy sector contrary to the porcine livestock industry where external biosecurity scores (65/100) are on average higher than the internal biosecurity scores (52/100).

The expert panel considered external biosecurity to have a larger effect on the biosecurity level of a fish farm than internal biosecurity. Within the category of external biosecurity, the following subcategories and their corresponding preventive measures were considered to be the most important: Off farm movements of live animals, entrance of visitors and personnel, and infrastructure and biological vectors. Within the category of internal biosecurity, the subcategory cleaning and disinfection was identified as the most important in preventing diseases.

Total of sixty seven marks was allocated for all external biosecurity measures and each subcategory (measure) was divided into several sub measures. Subsequently, total of thirty three marks was allocated for internal biosecurity measures by the panel of experts. Subcategory was given a score out of sixty seven in external measures and out of thirty three in internal measures. Each sub measure was allocated maximum of 25 marks using the risk matrix considering the likelihood of spreading a disease by the transmission route and the severity of disease.

The procedure followed in obtaining the final score of the internal and external biosecurity began with the allocation of a score between 0 and 1 for each question, 0 for total absence of preventive measure or full presence of risk and 1 for full presence of preventive measure or total absence of risk. To obtain the relative result of the question, the

said score was then multiplied by the weightage given to the specific question. This was followed by summation of the results of each question under the given subcategory and then dividing it by the maximum score obtained in the said subcategory. To derive the subcategory score, the above calculated proportional result of the subcategory was multiplied by the weightage assigned to the given subcategory and the final score of the internal and external biosecurity is the sum of the scores obtained by each subcategory of internal and external biosecurity. This method was adhered to ensure that the scoring system is risk based and weights are included both at the level of the subcategories as well as at the level of the individual questions.

Sub category score calculation for each measure using risk matrix

Lewerin, et al., conducted a study to formulate a risk assessment model structure considering the likelihood of the introduction of a specific disease agent *via* certain contact. Severity of the effect of contact was calculated by multiplying the likelihood of the introduction of disease and severity of the effect of biosecurity measure. Risk assessment is a practical approach to ensure the consideration of severity of the consequence of the transmission route and the likelihood of occurrence of disease. The report on Malaysian pineapple biosecurity was the most recent finalized report available to review the application of the risk matrix method successfully.

The risk matrix method was used to conduct the risk analysis of pathogen transmission route considered in external and internal biosecurity measures in this study. Likelihood of the disease occurrence by the transmission route and the severity of a risk event are the two constructs considered in risk matrix.

As explained below, the process of creating the risk matrix consisted of three steps.

Step 1: Scaling the likelihood and severity of a risk event (*i.e.*, a risk factor) measuring on the five point likert scale;

- Very low.
- Low.
- Moderate.
- High.
- Very high.

Step 2: Preparing the risk index output by using the severity impact and the likelihood of the event. Magnitude of a risk factor “R” is identified using the equation given below.

$$R=L \times S$$

Where;

R=Magnitude of the risk,

L=Likelihood of the event,

S=Severity of the event.

Step 3: Preparing the standard risk matrix by dividing the probability of occurrence and severity of an event into five levels as 1-5 and then mapping the risk matrix.

Magnitude of the risk matrix is prepared using an equation based on the scale of probability, the scale of severity and the data collected from experts.

The magnitude of a risk is divided into three categories; namely, 1-4 negligible, 5-12 moderate and 15-25 critical (Table 1).

Severity	s5=Very high	5	10	15	20	25
	s4=High	4	8	12	16	20
	s3=Modarate	3	6	9	12	15
	s2=Low	2	4	6	8	10
	s1=Very low	1	2	3	4	5
		L1=Very low	L2=Low	L3=Modarate	L4=High	L5=Very high
		Likelihood				

Table 1: Risk matrix used to calculate the sub category measure scores.

In addition to considering the level of efficiency in disease transmission of a specific transmission route, the scoring system also takes into account the frequency at which the transmission route occurs. A particular transmission route will pose a substantial risk, when indirect contact takes place at a higher frequency, even if the probability in transmitting the given disease is relatively smaller. Laanen, et al., and under such circumstances there will be a higher estimated effect.

Different disease transmission routes and acquired scores from the literature survey and expert panel

External biosecurity: Source of purchasing of brood stock or growers to the farm. When new animals are introduced to the farm, the probability of a new pathogen entering the farm is extremely high. This happens most of the time when the brood stock replacement is taking place by purchasing new fish to the farm.

The higher the frequency of new entries, the higher the likelihood of a new pathogen entering to the farm and on the other hand, the higher the replacement rate, the more difficult it is to maintain herd or flock immunity against the endemic farm pathogens. Risk associated with this transmission route can be minimized by reducing the frequency of purchasing new brood stocks to the farm, in addition to maintaining internal brood stock and proper quarantine practices.

In such case, well planned quarantine set up is suggested as the most effective way to minimize the risk of introducing new pathogens. Testing for the susceptible fish diseases acquired from the new batch, possible vaccination along with the good quarantine management practice are the methods suggested by the experts to overcome such a risk. Quarantine unit must be designed by blocking the connection with the main farm. At the same time all in and all out system must be practiced to prevent transmission of pathogens to different other fish

species and batches. This involves bringing animals in as a group from only one original source population and maintaining them as a group throughout the quarantine period. It prevents exposure to other pathogens not currently in that population. Ideally, no new animals should be added to a group currently in quarantine. All in all out quarantine can be applied within an entire facility, room or system.

Direct contact is one of the most common routes of disease transmission in aquaculture. This involves the transfer of disease causing agents through contact with infected fish. Entry may occur through the skin, open wounds, mucous membranes, or gills. Infectious microorganisms can be found on the mucus layer of fish, also it could occur from seeping lesions. Some pathogens spread from

female fish to her eggs (vertical transmission). There are carriers of vertical transmitted or horizontal transmitted pathogens acquired from new fish. According to the findings of different studies purchasing animals from different farms found to pose a greater risk of introduction of pathogens to the farm.

According to the findings of a study done on aquaculture in Egypt, only 3% of the farms strictly follow the biosecurity practices in fish farms after stocking new fish, for example, keeping fish in separate quarantine tanks to prevent them from getting infected (Table 2). Study revealed that the low level of biosecurity practices result in higher mortality.

Transmission route	Different strategies of biosecurity	Citation
External biosecurity consideration Purchasing new fish to the farm	Bringing from the same farms. Purchasing new animals. Quarantine facilities and all in all out system. Disinfection of transport vehicle. Frequency of purchasing new fish.	Hege, et al., Villarroel, et al., Yanong, et al., Shimaa, et al., Baraitareanu, et al., Yanong, et al., Shimaa, et al.
Feed and water supply	Storage facility to fish feed. Water storage facility. Water supply from a clean source. Quality feed.	Villarroel, et al.
Removal of waste water and dead fish	Water disposal to a pit. Gutters always clean without accumulation of water. Proper disposal of dead fish.	Damianns, et al.
Visitors and farm workers hygiene	Visitor should make a prior notification to enter the farm. All farm workers and the owner abide by the rules accessing the farm. Farm specific clean clothes and shoes are available. Hand disinfection facility. Visitor access limited. Workers not rearing fish at home. Separate workers for each section.	Noremarket, et al., Baraitareanu, et al., Kapperud, et al., Refegier-Petton, et al.
Supply of materials	Materials shared with farms. Shared materials disinfected.	Amass Baysinger, et al.
Biological vector	Access of fish to outside of the farm. Feed stored securely to prevent rodents and other pests accessing. Wild birds enter the fish farm. Bird and vermin proof air inlets. Fenced farms. Pet access is prevented.	Yanong, et al., Baraitareanu, et al.
Location of the farm	Water is not stagnant. Distance between the nearest farms is more than 500 m. Wind or waste water not coming from other farms.	Truscutt, et al., Bradburry, et al.

Table 2: Literature references of different external biosecurity strategies against different pathogen transmission routes.

Feed and water supply

Feed is an important source of disease transmission, feed itself does not generally pose a risk of transmitting diseases unless different pathogens contaminate and survive on food ingredients and feedstuff. There are numerous feed contamination mitigation practices available for consideration, for example, preparation of uncontaminated feed by monitoring the hygiene of raw materials and the feed mills, proper storage of feed, adding prophylactic treatments to the feed formula etc. Feed can act as a mechanical vector, thus as a source of infection. Contamination of feed could occur during improper storage, production and transport. Infected food can introduce pathogens to the gut. Feeding food caught in the wild, e.g. *Daphnia*, or dead animals (died either through a disease or “natural” causes) should be avoided as this carries a high risk of introducing infective agents to the gut. It is necessary to be extra careful when feeding fish with live food. Wild harvested live foods may harbor parasites or other harmful organisms that can cause disease in fish. It has been well proven that pathogens can be easily transmitted to fish through low quality feed. Many incidences of disease outbreaks are the result of improper feed formulation, excessive feeding rates or variation in sizes of stocked fish. It is important to purchase live food from a reputable source. Feed intoxications can happen in aquarium settings. The most common of these is a result of feeding fish with food contaminated with the aflatoxin produced by *Aspergillus* flavors, a mold that grows on the feed. When this aflatoxin is eaten, it causes rapid growth of tumors and a high death rate. Moldy feeds should always be discarded.

Water used in the fish farm is a strong source of pathogen introduction. Reliable source of continuous water supply with storage tank is essential to ensure the water hygiene in the farm. Water systems, tanks and pipes should be disinfected and cleaned regularly as biofilms can be a strong source of bacterial pathogens. Therefore, bacteriological quality of the water should be checked regularly. At least common water treatment techniques such as UV filtration or application of chemical should be practiced.

Water including the packing water is important potential source of infections. It is important to have an overhead tank as the water reservoir, thus avoid contamination *via* dust, wild birds or rodents and chemicals. Regular monitoring of the water source and packing water is recommended.

Water sources can transfer disease causing organisms. Infected fish can contaminate the water sources they are living in. Contamination could occur from the urine, feces, reproductive fluids and mucus of infected fish. Movement of this contaminated water during the transport of fish can spread pathogens to new locations. A few fish pathogens (e.g., *Ichthyophthirius multifiliis* (ICH)) have been found to spread *via* aerosols, sprays or splashes between tanks. This is less common and typically requires sources to be in closer proximity.

Certain water quality parameters are known to cause serious problems to fish, especially when they are living under intensive farming conditions. These problems cause discomfort to fish, heavy mortalities and in addition, factors such as the fish species, the time and level of exposure and the synergic effect of other coexisting stressful conditions, play an important role towards this. Further, when these parameters are outside the preferable for each fish species range, they can induce stress to fish, compromising their immune system and making them vulnerable to many opportunistic pathogens.

Unusual mortalities have been observed depending on the bad quality of water sources where irrigation canal and surface water is

used as the main water source. Findings of the study revealed that the quality of the water source is significantly associated with the occurrence of unusual mortality.

Visitors and workers hygiene

It is important to limit the number of visitors and at the same time workers also should be limited, at least in each section as pathogens can be transmitted between sections or compartments.

Once the visitor is allowed to enter the premises, he or she must be registered in the visitors' registry. There should be thorough investigation on his/her farm visits within last 24 hours-48 hours. Real evaluation of the liability of spreading different fish pathogens may exceed the above mentioned time period since survival of various pathogens in the contaminated environments may vary. Nevertheless, the biosecurity barriers established at the entrance like good standards in hand washing facility, outwear and boots changing facility, showering facility may minimize the possibility of most of the pathogens entering the farm through visitors.

Supply of materials

In addition to persons and vehicles, instruments can also serve as disease transmission vectors. To prevent the transmission of pathogens from one herd to another, it is wise to use section or tank specific instruments. To prevent contaminated equipment from being brought into the farm by contractors (ladders, tools, and so on). It is advisable to ensure the availability of this type of equipment at the farm enabling the contractors to use farm specific equipment.

Fomites are inanimate objects that can transfer pathogens between rearing areas or production sites. These items become contaminated when contacted with infected fish or contaminated water sources. Examples include equipment, such as nets, buckets, siphon hoses, any equipment that comes in contact with fish (especially sick fish). Fomites also include footwear or clothing worn by fish handlers, or vehicles (e.g., hauling trucks/tanks) that may be used to transport potentially infected fish or contaminated water.

Infrastructure and biological vectors

Some parasites cannot complete their life cycle unless a secondary host (a snail for instance) is present. The continuous prevention of contacting the vector/disease/host life cycle will keep the stock disease free. *Centrocestus* spp. needs an intermediate snail host to complete its life cycle. With good biosecurity management the parasitic infestations can be controlled by expelling the intermediate host in an aquaculture set up.

For instance, as is the case in a number of diseases, if a snail is the intermediate host then removal of the snail breaks the infection cycle and prevents the outbreak of disease. For an example presence of birds and snails facilitate infestations by digeneans, requiring fish and snails as intermediate host and the aquatic birds as the final host. Digenean trematodes life cycle is complex as their hosts belonged to all the vertebrate groups which represent most of the terrestrial, fresh water and marine invertebrates.

Less commonly, fish diseases may be spread by vectors. Vectors are living creatures, such as fish preying birds that can spread disease pathogens. These animals may transfer fish diseases between locations by carrying the pathogen on their body or feet, or by dropping infectious dead fish or fish parts at other locations.

Rodents may also carry fish pathogens on their body or in their feces or urine, contaminating the environment or fish feeds. Domestic animals (such as pets running around the farm) may also serve as cross contamination mechanisms for some pathogens. People can serve as vectors by transferring pathogens to fish during handling.

Pathogens can be introduced on the farm by rodents, wild birds, and insects but also *via* pet animals and other farm animals. Rodents may serve as biological as well as mechanical vectors of pathogens. They may spread infections both within and between neighboring farms. Lister, stressed that none of the equipment, weeds, or waste should be stored against the outer walls and feed should be stored in vermin free place to prevent rodents from nesting in the vicinity of the farm. Wild birds are associated with *Mycoplasma* spp., *Campylobacter* spp., *Salmonella* spp., *Yersinia* spp., and *Mycobacterium avium*.

Leeches spread carp trypanosomiasis between fish. Without treatment, infected fish will remain infectious for long periods and the disease can be spread again if leeches find their way back into that system. Full eradication can only be completed if the fish are treated and the leeches are eradicated.

Ventilation holes need to be closed with wire mesh to prevent rodents from entering. Entire farm needs to be covered with a net to discourage wild or migratory birds. Insects can act as a vector for a variety of pathogens.

Location of the farm

The farmer has no real control over the location of his ornamental fish farm even though he has established in the right location as the outside environment may change with the time. However, this does not undermine the fact that several important pathogens can be transmitted through air. Density in the vicinity of the farm is an important factor for those pathogens for which transmission is density

dependent. A good example is some viral diseases, for which it has already been shown that this pathogen can spread as fomites by wind and under favorable conditions it can remain infectious for a long time. To reduce the likelihood of airborne transmission between farms, the distance to the nearest neighbor should be at least 500 m and preferably >1 km. When farms are closer to each other, attention should be paid to the predominant wind direction.

Internal biosecurity

Disease management: Disease management consists of vaccinating susceptible animals, a strict destroying policy, removing dead fish from the tanks, and controlling the stocking density. Infective stages of some pathogens can leave the host and travel through the water directly to their next host. Normally, the higher the stocking density is, the more likely that the pathogens will spread through direct contact. The fish stocking density influences the severity of a disease outbreak. A high stocking density induces stress, which results in an increased susceptibility to infections and an increased excretion of pathogens. Many infected animals in a small area entail a sharp increase in the infection pressure. In addition to the risk of transmission, stocking density also influences production results.

It is also important to pay attention to establish a sectioning plan to consider the disease transmission of different species of fish within the farm. Unlike cattle, pig or poultry farms it is very difficult to manage the ornamental fish farm without keeping different age groups in the same establishment. The usual recommendation is to establish a work flow from younger to older. Sectioning of farm as the nursery unit, growing unit, brood stock unit and adult stage unit ready for harvesting and quarantine unit would be beneficial for this purpose. Persons working at each unit should not enter into other sections (Table 3).

Transmission route	Biosecurity strategy	Citation
Internal biosecurity disease management	Acceptable stocking density. Prophylactic treatment. Health management programs.	Sims, et al.
Cleaning and disinfection	Disinfection cleaning after each production cycle Foot bath available. Clean cloths available. Clean boots available. Vehicle bath available. Hand washing facility.	Meroz, et al., Felix, et al., Shimaa, et al.
Materials and utensils between compartments	Materials disinfected between compartments. Protocol for disinfection is available. Clearly recognizable material code between compartments.	Shimaa, et al.

Table 3: Literature references for different internal biosecurity strategies against pathogen transmission routes.

Vaccination is an important control measure for many diseases that are ubiquitously present. Vaccination can consider as an animal welfare tool, apart from reduction of losses due to morbidity and mortality. Morbid animals need to be destroyed as there is a high risk these animals could spread the pathogen to other susceptible fish.

Cleaning and disinfection

Cleaning and disinfecting is of great importance for the control of diseases. It is important to prevent fish from coming to contact with debris from the previous batch. Some pathogens can survive for a long time in the environment without the presence of animals. Therefore,

the following steps of the complete cleaning and disinfection protocol should be carried out between two production cycles: Dry cleaning, wet cleaning, disinfection, vacancy period, and monitoring the efficacy.

Not only may the interior of the tanks, but also the environment around the farm including gutters and the ground form a potential reservoir for several pathogens. Therefore, the hardened environment around the stables needs to be cleaned as well. The findings of latter study also show the importance of taking hygienic measures before entering the farm.

Studies done to find out unusual mortalities in fish farms found that majority of farmers (33.3%) dried tanks between production cycles. Nearly 23% farmers practiced tank drying using lime and 17% with cleaning nets. A total of 17% farmers practiced drying, liming and cleaning nets while only 4.6% practiced cleaning nets alone. 34% farmers are not practicing any of the above procedures and the researchers have found there is a significant association between the mortalities and the lower applications of biosecurity practices.

The facilities should contribute to reduce the transmission of diseases or, at least, must not facilitate their spread. A very basic aspect to start with would be its design. In poorly designed or poorly planned farms it is relatively common for animals to move between different sections for loading, unloading or between production phases so that animals of different ages can have contact. Likewise, it is important that the facilities allow a correct organization of work and, to a certain extent, contribute to respect a separation between the different ages present on the farm. This can be achieved with physical barriers such as doors, foot baths, or intermediate areas for hand washing and changing boots.

However, all these barrier measures tend to hinder work routines. Sometimes the different areas can be painted with different colors and clothes and boots of the corresponding colour can be used to make it more difficult to violate the rule of non-contact between different stages of production.

Disinfection and cleaning are considered as basic elements in hygienic measures. Felix, et al., through the findings of their studies highlighted that a rapid onsite cleaning and disinfection regime is a proactive strategy to control the spread of microorganisms such as *Vibrio* species colonizing the fish farms.

Materials and measures between compartments

Equipment that is used can also cause the spread of pathogens. Catching nets, syphoning tools can easily be contaminated with infectious material. It is therefore recommended to use different equipment in different sections and make sure that this equipment is clearly recognizable (different colors) to avoid moving equipment from one section of the herd to another. The same rule can be applied to clothing, for exactly the same reason. Also, washing and disinfecting the hands between sections and tanks reduces the risk of transmission of pathogens.

Quantification of the biosecurity level

Based upon the different weights given by the expert panel to each biosecurity measure and (sub) category, a final weighted and risk

based score is calculated. To obtain this score, each answer to a specific question receives an individual score between 0 (=total absence of preventive measure or full presence of risk) and 1 (=full presence of preventive measure or total absence of the risk). This score is subsequently multiplied by the weight of the specific question to obtain the relative result of the question. Next, all the results of the individual questions within a subcategory are summed up and divided by the maximum score of the given subcategory. This proportional result of the subcategory is then multiplied by the weight of the subcategory to obtain the subcategory score. The final score of the internal and external biosecurity is the sum of the scores obtained by different subcategories. The overall biosecurity score is the sum of the external and internal biosecurity score. Due to the different relative weight, the external biosecurity score counts for 67% and the internal counts for 33% in the total biosecurity score. For the ease of interpretation of the results, category and subcategory scores are recalculated each time to a score on 100 and presented as a percentage in the reports.

Reporting of the biosecurity level

The questionnaire was filled in the field level based on the responses to personal interviews. The questionnaire can be filled in online, simultaneously by the farmer or by the veterinarian. Based on the answers given in the questionnaire, a farmer obtains a score between 0 and 100 for both external and internal biosecurity and the corresponding subcategories. All scores of the different subcategories were calculated immediately after completion of the questionnaire.

Collection of data

All data were collected between June 2021 and June 2022 through a personal interview at the farm. All fish farms were visited by the researcher with a view to minimize interviewer bias and to ensure inter farm comparability. After the interview was conducted and the questionnaire was filled in, all sections were visited and photographs were taken. The inspection of the different section was performed to allow a comparison of the answers given by the farmer and to observe the present situation in the fish farm. If the interviewer noticed that the given answers on the questionnaire did not tally with the real situation of the farm, the farmer was notified and the given answers were changed. Subsequently, a report was prepared according to the scores describing the current biosecurity management at each sub category level. Additional information was given on the importance of practicing biosecurity measures in the farm and on correcting the loopholes.

Results

Results of the scoring system

The majority of the questions were easily understood by the farmers, and only some minor vocabulary adjustments were made to improve the clarity of the questions (Table 4 and Figure 1).

Different biosecurity scores in average

	Average score of the farmer	Standard score
External biosecurity	3.81 (26%)	15
1. Purchasing new fish to the farm	4.3 (35.8%)	12
2. Feed and water	1.16 (16.57%)	7
3. Removal of waste water and dead fish	1.15 (12.7%)	9
4. Visitors and farm workers	3.58 (51.1%)	7
5. Material supply	2.73 (45.5%)	6
6. Biological vectors	3.5 (31.81%)	11
7. Location of the farm		
Internal biosecurity	5.42 (41.69%)	13
1. Disease management	3.05 (25.41%)	12
2. Cleaning and disinfection	2.5 (31.25%)	8
3. Materials and measures between compartments		

Table 4: Average scores of participant farmers for different biosecurity measures.

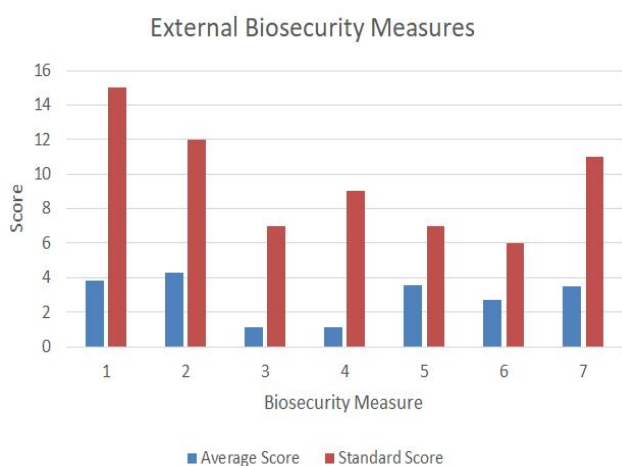


Figure 1: External biosecurity measures comparing the average scores acquired by the farmer with the standard scores given by the experts.

Within the category of external biosecurity, the following 3 subcategories had the lowest average scores acquired by ornamental fish farmers: Visitor and workers hygiene, waste water and dead fish removal and purchasing of new fish. In the subcategories of the external biosecurity, feed and water supply, material supply and biological vector control obtained the highest average scores. Within internal biosecurity, disease management obtained the highest score, whereas cleaning and disinfection had the lowest score comparing the standard scores of each category. Even considering the highest average values obtained by the farmers, which are far lower than the standard scores (Figure 2).

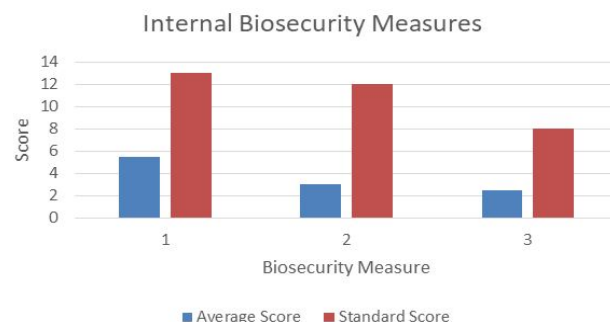


Figure 2: Internal biosecurity measures comparing average score acquired by the farmers and the standard scores given by the experts.

When comparing the average scores obtained by the farmers for the each category, internal biosecurity scores have reached closer to the standard values of each category.

The obtained results are also displayed graphically in a bar chart allowing rapid visual identification of any bottlenecks in the biosecurity management at the farm. The different axes of the bar chart represent the associated subcategories within external or internal biosecurity. Every score obtained by the farmer for a specific subcategory is plotted on the related axis. Subsequently, all plotted average farmers scores can be compared to the standard scores.

The maximum biosecurity score acquired by the farmer is 63% while the minimum is 5%. According to the results, farmers having higher education level acquired highest scores in biosecurity levels where as the farmers with lower levels of education gained lower scores.

Discussion

This study has developed a scoring tool for the quantitative evaluation of biosecurity of ornamental fish farms. The system could identify the biosecurity status as well as biosecurity gaps present in the farm. The scoring system includes very simple calculation which could help the veterinarian and eventually farmer to identify the gaps as well as to implement the biosecurity measures in the farm.

Despite the fact that the scoring system and the associated weights are based on a thorough literature study, previous comparable exercises in pig production and poultry production and the opinion of an expert panel, the attributed weights remain a subjective estimation of the importance of different preventive measures. However, the scoring system can be seen as a valuable tool to monitor the biosecurity level of ornamental fish farms over time. Different fish farms can easily be compared with one another and each farm can be followed up over time using the same scoring system. If the biosecurity scoring system is used throughout the country, the biosecurity level could be mapped out and high risk areas in which the diseases could spread can be identified. This may be helpful in case of epidemic disease outbreaks, thus making target surveillance possible.

This study has attempted to develop a risk based quantitative tool to measure the biosecurity level at fish farms in a standardized and reproducible manner. This scoring system can be used to quantify the biosecurity at farm level by considering almost all the aspects of biosecurity measures. In contrast to other biosecurity questionnaires in which no weights were given to the different measures, it is now possible to differentiate between farms with high and low biosecurity based on the type and application of biosecurity measures as the researcher mentioned in the previous section of this article. The values obtained by the research are very much valid as the scores have been weighted and the bottle necks of the biosecurity management are highlighted. By comparing the results of the farm to the national averages, a farmer can benchmark his status. Benchmarking of results has already proven to increase the awareness of social issues (e.g., antimicrobial use in livestock) and to stimulate farmers to improve the current farm situation on their farm.

Significant variation in biosecurity scores was found between different farms indicating that there is ample room in this sector for improvements.

In general, the internal biosecurity scores were higher than the external biosecurity scores in studies conducted in dairy sector Gelaude, et al. contrary to the porcine livestock industry where external biosecurity scores (65/100) are on average higher than the internal biosecurity scores (52/100).

A study conducted by Limbergen on European conventional broiler production found internal biosecurity score (mean 76.6) to be better than external biosecurity score (mean 68.4). There was a variation between the mean biosecurity scores for different member states, ranging from 59.8 to 78.0 for external biosecurity and from 63.0 to 85.6 for internal biosecurity. Sub category of visitors and staff scored the lowest biosecurity levels compared to the standard scores which suggested that better education of broiler farmers and the staff will help to improve the broiler farm biosecurity in Europe.

Similar study conducted by Daamians to assess the biosecurity in veal, beef and dairy farms in Europe found that, for all production systems both internal and external biosecurity to be at lower level. The bio check tool was used to assess the scores and this resulted in lower mean total biosecurity scores of 39.7 points for veal (SD=7.4), 44.3 for beef (SD=8.4), and 48.6 points for dairy farms (SD=8.1), out of a maximum of 100 points. "Health management" subcategory was observed as the lowest subcategory in all three production systems. This evaluation was important in benchmarking and in comparing all three types of farms in the area and in providing herd specific advices for improvement of biosecurity loopholes.

Based on the views of experts and the literature survey carried out, the external biosecurity score weightage is higher than the internal biosecurity score weightage in the ornamental fish industry in Sri Lanka. The average scores obtained by the farmers were far lower than the weighted standard scores and the internal biosecurity scores were comparatively higher than the external biosecurity scores on average. This difference between the external and internal biosecurity scores resulted due to the fact that there are less preventive measures for internal biosecurity when compared with the external biosecurity at ornamental fish farms in Sri Lanka. Therefore, high scores reaching the maximum score of 100 (hundred out of hundred) can be more easily obtained for internal biosecurity category.

Since the Sri Lankan ornamental fish industry concentrates mainly on export market, production is mainly determined by the legal and private requirements of the importing country. So far the domestic exporters have been successful in providing good quality and healthy fish to the world with zero complaints. As the sector is still robust in competing with other exporters in the world, it is important to take every possible step to mitigate the threat of spreading endemic or exotic diseases, which could have a detrimental impact on the whole industry. As the findings of this study suggests, there is more room for most of the biosecurity measures to be improved, this innovative tool will allow the farmers to observe their farm biosecurity levels, in a quantitative manner. While health standards, hygiene, traceability, social and environmental requirements are comparatively new, marketing standards for ornamental fish for the EU market have been in place since the early 1970 and the new conditions were imposed in 2014 for Australian exports. Other countries tend to amend the health standards, hygienic requirements, and traceability, social and environmental standards with the time to overcome different issues faced by the people engaged in live animal international trade.

According to several interviewees, Australia and the EU countries require the highest quality standards. Most retailers in these countries demand different health standards requiring special certificates other than the zoosanitary certificate issued by the department of animal production and health.

Conclusion

In Sri Lanka, the number of producers applied for registration has increased tremendously since 2020 and as at present out of 70 exporters, only 15 engage in production. It is observed that some of the producers have given up the ornamental fish breeding activity as the buyers started placing more orders from them. The registered exporters producing ornamental fish can supply maximum of 140,000 tails per month. Exporters who do not breed ornamental fish tend to purchase fish for their buyers on demand and they collect fish from different suppliers scattered all over the country. The most important factor to be taken into account is that the exporters that produce fish in their own farms are monitored regularly on their veterinary status by the department of animal production and health. The exporters that collect fish from different breeders are checked or regulated, only if the exporter declares the details of the fish suppliers to the department of animal production and health. This problem will be solved sooner as the department of animal production and health is working on mapping of ornamental fish farms scattered all over the country.

This innovative tool allows not only to study the biosecurity levels at farms, in a quantitative manner, but also the relationship between biosecurity, health, and production characteristics, similar to the way

this has been used for pig production, poultry and dairy production systems in other countries.

According to the knowledge of researchers, Sri Lankan ornamental fish industry biosecurity levels in the ornamental fish industry in Sri Lanka have not been systematically studied at the national level, also biosecurity scores at the national level have not yet been calculated. Thus, the biosecurity assessment tool developed in this study can be used to calculate the national average of biosecurity standards of the ornamental fish farms in Sri Lanka. This should be further followed up through the modification or expansion of the existing measures at farm level. Managing protocols must be evaluated and described at each step together with the training of the farm staff and the professionals that serve in this sector.

As the biosecurity has become an essential element of intensive farming systems, avoidance of the introduction of new pathogens and effectively controlling of their spread will contribute to increase the wellbeing of fish industry too. A better knowledge of the epidemiology of the fish diseases will contribute towards designing better biosecurity programs like quantitative assessment methods. Findings of a study of this nature is immensely important to the stakeholders of the industry as this has produced an objective quantitative assessment methods to permit precise selection of biosecurity measures and thereby to carry out proper evaluation of their impact in preserving the health of fish in Sri Lanka. Collaborative approach with other branches of science like sociology and psychology may help to implement a better biosecurity plan.

Limitations

As the respondents were from different educational backgrounds, there were variations in some of the answers provided to the questionnaire, the result of inconsistency in theoretical and practical knowledge of farmers.

Researcher developed the same questionnaire in an online platform and attempted to obtain answers from the ornamental fish farmers, nevertheless, the effectiveness of the attempt could not be assured as the respondents were not though in ICT knowledge.

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