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A Review of Fish Diseases in the Egyptian Aquaculture Sector. Working Report.

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1. OVERVIEW

Aquaculture is used to produce fish and shellfish for markets under controlled or semi-controlled conditions. Fish must be maintained at densities that greatly exceed those typically found in nature. Regardless of the culture system used (e.g. ponds, raceways, reuse systems, cages), it is imperative that the culturist maintains an environment conductive to good fish health. However, fish farming conditions are often conducive to the spread of disease.

Fish Diseases may be subdivided into:

- *Infectious diseases,* caused by pathogenic organisms present in the environment. They are mostly contagious and treatment may be necessary to control the disease outbreak.
- *Non-infectious diseases,* caused by environmental problems, nutritional deficiencies, or genetic anomalies. These are not contagious, usually cannot be cured by medications but rarely happen and are best prevented and controlled by provision of good water quality and good management.

Infectious diseases are more prevalent and broadly categorized as bacterial, parasitic, fungal, or viral diseases and usually associated with high mortality and morbidity rates with broad negative impacts on farmers, consumers and the environment.

The present study reviews infectious diseases among fish in Egyptian aquaculture and their impact on fish and human life, as well as the various interventions that have been used to attempt to prevent and control these diseases. Although, a considerable amount of research has been carried out into fish diseases in the Egyptian aquaculture sector, we focus on investigations that have been carried out since 2000.

2. SOURCE AND MODE OF INFECTION

The sources and modes of infection among fish are variable, as fish disease is rarely a simple association between pathogen, a host fish and an environmental problem. Other stressors, such as poor water quality often contribute to the outbreak of disease and the complexity of the challenge. Many pathogens are either normal inhabitants in or on fish or saprophytes present in soil or water or invertebrate hosts, such as snails or crustaceans. The majority of infections are stress related. The transmission of infection to fish occurs through direct and indirect exposure of cultured fish to pathogens, which is facilitated by poor fish health management. The

mechanisms by which fish diseases are transmitted generally including a mixture of the following: contaminated water supply, infected eggs or fish stocks and/or contaminated culture facilities, together with environmental conditions associated with the fish culture practice (air, ponds, soil, equipments, feed, pollutants, etc.).

2.1 Bacteria

Bacteria are responsible for many diseases and heavy mortalities in farmed fish. Most of the causative micro-organisms are naturally occurring saprophytes, which utilize the organic and mineral matter in the aquatic environment to grow and multiply. It has been shown that the normal bacterial flora of fish reflects the bacterial population of the water in which they swim. The majority of fish pathogenic bacteria are short, Gram-negative rods belonging to the families Enterobacteriaceae, Pseudomonadaceae and Vibrionaceae. Typically they cause septicemic and ulcerative disease conditions. The long, Gram-negative, myxobacteria of the family Cytophagaceae, which are not recognized as pathogens of warm-blooded animals, may also cause heavy mortality in fish stocks. Gram-positive micro-organisms, including a few that are acid-fast, are less frequently encountered, but can cause severe losses in certain species of fish under particular conditions.

During 2000, severe mortalities and morbidities were seen among cultured Nile tilapia (*Oreochromis niloticus*) in several large freshwater fish farms in Egypt (see Table 1). Laboratory studies revealed the presence of *Aeromonas hydrophila* in 70% of fish examined. The recovery rate of *Aeromonas hydrophila* from skin, muscle, kidney, spleen and liver tissues were 53%, 35%, 65%, 63% and 60% respectively[(1)]. Mortalities in both tilapia sp. and mullet sp. due to bacterial infections also occurred in several farms at Dakahila and Sharkia Governorates, where laboratory investigations isolated *Aeromonas hydrophila* and *Flexibacter columnaris*[(2)]. Moreover, *Vibro anguillarum,* as an economically damaging infectious disease, was recovered from 62% of clinically affected Nile tilapia. The percentages of isolation from skin lesions, muscles, kidney, spleen and liver tissues were 35%, 22%, 60%, 48% and 43%; respectively[(3)].

During 2001, columnaris disease was reported among *Oreochromis niloticus* and *Clarias lazera* cultured in the Abbassa Fish Farm, Sharkia. Identification of the isolates revealed *Flavobacterium columnare* and *Cytophaga spp*[(4)] (Table 1). *Pseudomonas fluorescens* was also isolated from carp in the Abbassa Fish Farm, with a prevalence rate of 23%[(5)]. *Yersinia ruckeri* (9.3%) was isolated from both apparently healthy and diseased cultured *O. niloticus* (8.3% and 12.4% respectively), and *C. lazera* (7.0% and 10.8%). In *C. auratus* and *C. carpio,* the incidence in apparently healthy fish was 3.8% and 2.5%, respectively[(6)].

Table 1. Common bacterial infections among freshwater fish.

Year of record	Bacterial pathogen	Fish species affected	Site
2000	*Aeromonas hydrophila, Flavobacterium columnare, Vibro anguillarum*	Nile tilapia, mullet sp., *Clarias* catfish	Dakahila and Sharkia
2001	*F. columnare, Pseudomonas fluorescens, Yersinia ruckeri*	Nile tilapia, *Clarias* catfish, carp, goldfish *(C. auratus)* and common carp	Abbassa
2002	*Pseudomonas fluorescens, Streptococcus iniae*	*Oreochromis niloticus*	Ismailia, Sharkia, Fayoum
2003	*KIebsiella pneumonia, Enterococcus faecalis*	Nile tilapia	Kafr EI-Sheikh
2004	*Pseudomonas fluorescens, P. aureginosa, P. anguilliseptica, P. pseudoalkaligenes*	Nile tilapia, African catfish, silver carp and grey mullet	Kafr EI-Sheikh
2005	*Yersinia ruckeri*	Nile tilapia, common carp and monosex tilapia	Behera and Kafr El-Sheikh
2006	*Edwardsiella tarda, E. Ictaluri, Streptococcus faecelis, A. hydrophila* and *P. fluorescens*	Nile tilapia, common carp, African catfish, and grey mullet	Behera, Kafr El-Sheikh and Alexandria
2008	*F. columnare*	Nile tilapia	Behera
2009	*Enterococcus faecalis*, *Streptococcus iniae*	Nile tilapia	Kafr El Sheik

During 2002 *Pseudomonas fluorescens* was isolated from Nile tilapia cultured in duck-fish farms at Ismailia and Sharkia Provinces with prevalence of 8%[(7)] (Table 1). Seventy eight isolates of *Streptococcus iniae* were also recovered with an incidence of 86.7% from diseased Nile tilapia cultured in brackish water in Fayoum Governorate. The environmentally stressed fish showed a mortality rate of 73.3%, compared with a mortality rate of 46.6% in non-environmentally stressed fish[(8)].

During 2003, outbreaks of *KIebsiella pneumoniae* in 5 - 7 month old Nile tilapia were recorded in three farms in Kafr EI-Sheikh Governorate, with mortality up to 27.7%[(9)] (Table 1). *Enterococcus faecalis* was recovered from Nile tilapia and rearing pond water samples reached 43.3%, 30% .0% and 85%, 60%, 5% in extensively, semi intensively and intensively operating fish farms, respectively[(10)].

During 2004, *Pseudomonas* spp. was isolated from Nile tilapia and African catfish (*Clarius gariepinus),* silver carp (*Hypophthalmichthys molitrix*) and grey mullet (*Mugil cephalus*) that were being reared in seventeen commercial fish farms in Kafr El-Sheikh Governorate (Table 1). Seven of the seventeen farms examined suffered from high mortalities, ranging from 17.6 to 22.9%. Bacteriological examinations revealed 38 fish (36.9%) were infected with *Pseudomonas fluorescens*, 30 (29.1%) with *Pseudomonas aureginosa*, 19 (18.5%) with *Pseudomonas anguilliseptica* and 16 (15.5%) with *Pseudomonas pseudoalkaligene*[(11)].

During 2005, *Yersinia ruckeri* was isolated from Nile tilapia, common carp *(Cyprinus carpio*) and monosex tilapia from different areas in both Behera and Kafr El-Sheikh Governorates (Table 1). The mortality number and percentage in monosex tilapia were lower than in common carp[(12)].

During 2006, *Enterobactereacea* (11 strains of *Edwardsiella tarda* and 9 strains of *E. Ictaluri*) were isolated from Nile tilapia*,* common carp and African catfish (50 ± 2 g) that were cultured in Behera, Kafr El-Sheikh and Alexandria Governorates[(13)] (Table 1). *Streptococcus faecelis* bacteria was recovered from monosex tilapia and grey mullet from different areas in Behera Governorate[(14)]. In fish farms in Behera, Kafr El Sheikh and Alexandria Provinces, *Enterobactereacea (E. tarda and Yersinia* spp.) were isolated from of Nile tilapia, common carp, African catfish and grey mullet (50 ± 2 g) at an incidence of 34%, 24%, 50% and 20%, respectively[(15)]. *A. hydrophila* and *P. fluorescens* were isolated from tilapia and African catfish at an incidence of 50% and 16.9%, respectively, while each of *A. caviae* and *A. sobria* were isolated with an incidence of 20% and 12.3%, respectively[(16)].

During late summer of 2008, an outbreak caused mortality of about 15% among cultured Nile tilapia in a private fish farm in Behera governorate due to infection *F. columnare*[(17)].

During 2009, the Bacteriological examination of 120 fish samples collected from Kafr El-Sheikh Governorate (60 diseased and 60 apparently healthy fish) revealed the isolation of 26 *Streptococcus* isolates with an incidence of 43.3% from diseased Nile tilapia and isolation of 17 isolates, with an incidence of 28.3%, from the 60 apparently healthy fish. The serological examination of 37 selected isolates result in differentiation into 17 *Enterococcus faecalis*, 12 *Streptococcus iniae*, 5 *Streptococcus pneumoniae* and 3 untype-able strains[(18)].

2.2 Parasitic infections

Parasites are the most common cause of infectious diseases. There are both opportunistic and obligate parasites. Obviously, for the obligate parasite, it is to the parasite's advantage not to kill the host if it is to live and reproduce. So, we find numerous parasites in wild fish which cause very little problem. Problems occur when infected fish are brought into the laboratory or into an intensive culture situation. Not only are the fish unusually stressed but they are also usually crowded and the reproducing parasites are not dispersed as they are in the wild. The closer the proximity of fish to one another the greater the probability of infection and mortality. Only the major parasite problems in cultured fish are covered here. Parasitic diseases of fish are classified into protozoan, crustacean and helminthic diseases. Generally, most of the crustaceans are external parasites causing severe diseases while protozoans cause either external or internal diseases according to their habitats. The majority of monogeneans and annelids are external parasitic diseases, while the majority of digeneans cause internal parasitic diseases. Nematode, acanthocephalan and cestode infestations are in general internal parasitic diseases. Nevertheless, a number of parasites with larval stages in fresh water fish have a piscivorous mammalian carnivore as their normal final host and are able to infect humans because of low host specificity of the adult stage.

During 2000, encysted metacercariae were encountered in the muscles of cultured tilapia fish in Abbassa fish farm (see Table 2). After experimental infection, three Prohemistomatidae adult worms (*Prohemistomum vivax, Mesostephanus appendiculatus* and *Mesostephanus melvi)* were recorded[19]. Similarly, encysted metacercariae (EMC) were collected from Nile tilapia at Dakahlia, and after experimental infection, adult flukes were recovered and identified as *Prohemistomum vivax, Pygidiopsis genata, Procerovum varium* and *Haplorchis pumilio*[20].

During 2001, the prevalence of *Trypanosoma* infection was recorded in wild *Chrysichthys auratus* (42.3%) and African catfish (8%). The lowest infection was found in *Morymyrus kanumme* (3.5%) and *Bagrus bajad* (2.5%) while Nile tilapia and *Labeo niloticus* were free from infection[21] (Table 2). Other research studies were carried out on tilapia from three localities in Egypt, where 61.3% fish were infected with six different types of encysted metacercariae. *Heterophyid* metacercariae were reported from *Tilapia zillii* and Nile tilapia, haplorchid metacercariae were found in *T. galilae*, blue tilapa (*O. aureus*), *T. zillii* and Nile tilapia. *Clinostomatid* and *euclinostomatid* metacercariae occurred at the lowest percentage among *T. zillii*.

Table 2. Parasitic infections among freshwater fish in Egypt, 2000 - 2012.

Year of record	Type of Infection	Species affected	Site
2000	Encysted metacercariae	Nile tilapia	Sharkia, Dakahlia
2001	*Trypanosome,* Encysted metacercariae, monogenea, ectoparasites	African catfish, *Morymyrus kanumme*, *Bagrus bajad* and Nile tilapia	
2002	Ectoparasites, metacercariae	African catfish, Nile tilapia	Dakahlia
2003	Ectoparasites, monogenea, helminthes	Freshwater fishes	
2004	Ectoparasites	Nile tilapia, blue tilapia, *Tilapia zillii*, African catfish and common carp	Sharkia, Dakahlia
2006	Metacercariae, fluke trematodes and Cestodes	African catfish	Ismailia
2007	Ectoparasites	*Oreochromis* spp., *Clarias lazera,* silver carp, black carp and common carp	Behera, Sharkia
2008	Cleidodiscus aculeatus	Common carp	Sharkia
2009	*Trichodina mutabilis*, *Chilodonella hexasticha*, *Gyrodactylus rysavyi* and Hetrophyid metacercariae *Lernaea cyprinacea*	Nile tilapia Silver carp, grass carp and mirror carp	Giza Sharkia
2010	*Quadriacanthus clariadis*, *Orientocreadium* sp., *Polyonchobothrium* sp., unidentified encysted metacercariae	African catfish	Dakahlyia
2012	*Anguillicolacrassus crassus*	eel *Anguilla anguilla*	Alexandria, Sharkia and Dakahlia

Experimental feeding resulted in the recovery of the following flukes: *Prohemistomum vivax, Pygidiopsis genata, Heterophyes heterophyes, Phagicola mollienesicola, Haplorchis pumilio, H. taichui* and *H. wellsi*[22]. Moreover, a study carried out on *Clarias lazera* and *Synodontis schall* for the external and internal

parasitic diseases and revealed an infection rate of 59.73%. Infection among *Clarias lazera* represent 90.27%, while that of *Synodontis schall* was 6.09%. External parasitic diseases found associated with *Clarias lazera* included *Trichodiniasis*, *Cichlidogyrus* and *Gyrodactylus* while in *Synodontis schall* were *Gyrodactylus.* Internal parasitic diseases found in *Clarias lazera* were *Henneguyan psorospermica* and *H. lobosa,* beside adults of the trematode *Orientocreadium* sp., the cestode *Polyonchobothrium* sp.*,* the nematodes *Procamallanus* sp. and *Paracamallanus* sp. and blood parasites *Trypanosoma* sp. and *Babesiosoma* sp., while internal parasites in *Synodontis schall* were metacercaria of a *Prohemistomatid* and a nematode (P*rocamallanus* sp.*)*[23]**.**

During 2002 African catfish were examined in Dakahlia Province for parasites (Table 2). Forty percent were found to be infected. The skin showed *Trichodina fultoni* (21.2%), *Chilodonella hexastica* (11%), *Ichthyophthirius multifi* (2.5%), *Ichthyoboda spp.* (6.25%) and *Myxobolus dermatobia* (5%). Most infections were in the gills, which were infected with *Trichodina fultoni* (l3.3%), *Ichthyoboda spp.* (4%), *Henneguya branchialis* (16.2%) and Myxo*bolus spp.* (3.5%). All isolated protozoa were at greatest prevalence during winter, followed by spring[24]. A parallel study also revealed that the prevalence and abundance of the metacercariae of *Centrocestus* sp. (Trematoda: Heterophidae) were recorded on gills of Nile tilapia and revealed 19.5 - 98.46% infection rate[25].

During 2003, the prevalence of infection with *Ichthyobodo necator* in grass carp (*Ctenopharyngodon idella*) was 100% while that with *Capillaria larvae* was 50%, while, the prevalence of infection in Nile tilapia with a mixed infestation of *Trichodina spp*. and *Gyrodactylus spp*. was 100%[26] (Table 2). In the same year, seven freshwater fish species were investigated for helminth parasites. The infection rate was 48%: acanthocephala (14%), cestodes (16.22%), digenea (10.66%), monogenea (1.77%), and nematodes (6.22%) were recorded[27]**.**

During 2004, an investigation of entero-protozoan parasites in five fish species (Nile tilapia, blue tilapia, *Tilapia zillii*, African catfish and common carp) of farmed fishes at the Abbassa fish farm was carried out (Table 2). The results revealed an overall infection rate of 66.9%, which was represented by 62.3% in Nile tilapia*,* 56.5% in blue tilapia*,* 80.1% in *T. zillii,* 58.1% in African catfish and 50% in common carp*.* The protozoan parasites included *Eimeria aurati* (35.3%), *E. rutili* (4%), *Eimeria* sp*.* (11%), *Goussia* sp. I (34.2%), *Goussia* sp. II (2.6%), *Cryptosporidium nasorum* (47.2%), *Myxobolus nkolyaensis* (2.2%), *M. carassii* (2.2%), *M. pharyngeus* (9.2%), *Mixidium lieberkuehni* (1.1%), *Ceratomyxia drepanofjettae* (1.8%), *Entamoeba molae* (7%), He*xamita sp.* (7%) and *Trypanosoma tilapiae* (0.7%)[28]. A parallel study was carried out during the same year for the external parasites that infest freshwater fish,

mainly tilapia species (*T. zillii*, blue tilapia and Nile tilapia), African catfish, common carp and mullets collected from different aquaculture facilities in Sharkia Governorate. Twelve external parasite species were identified, eight of which were monogenetic trematodes (*Macrogyrodactylus congolensis, Cichlidogyrus tiberinaus*, *C. magnus, C. arthracanthus, C. euzeti* , *C. longicornis longicornis*, *C. thurstonae* and *Heterothecium dicrophallum*), two of which were protozoans (*Trichodina domergue* and *Henneguya branchialis*) and two crustaceans (*Learnea* sp. and *Ergasilus* sp.)[29]. Another investigation of parasitic infestation of Nile tilapia was carried out on private fish farms in Dakahlia Governorate. The total prevalence of parasitic infestation was 63.3%, while skin and fin infestations were 61.8 and *38.2%,* respectively. The infestation rate with *Trichodina, Chilodonella, Scyphidia, Apiosom sp., Icthyoborzecator, Gyrodactylus sp.* and mixed monogenea with protozoa were 20.7%, 8.9%, 13.8%, 3.3%, 2.9%, 7.8% and 6%, respectively. The prevalence of parasitic infestation in Nile tilapia was high in autumn (26.7%) and least during summer (13.3%)[30].

During 2006 a number of African catfish cultured in Ismailia Governorate were investigated for internal parasitic diseases (Table 2). The prevalence of infection was 73.80%. The infection rates varied with season; spring (66.66%), summer (83.05%) and autumn (81.36%) while the lowest level was during winter (63.15%). The infestation rate was determined; nematode (19.28%), metacercariae (27.85%), fluke trematodes (18.57%) and cestodes (8.18%). The parasitological examination of infested fish revealed adult trematodes from the intestine *(Afromacroderoides lazera, Orientocreadium lazeri and Astiotremma reniferum),* metacercariae from the musculature and liver *(Prohemistomatid metacercariae, Diplostomum tilapi* and *Cyanodiplostomotid).* Cestodes *(Polynchobothrium clarias)* and nematodes from the intestines *(Procamallanus laeviconchus* and *Paracamallanus cyathopharynx)*[31].

During 2007 the ectoparasites infesting some freshwater fishes (*Oreochromis* spp), *C. lazera* and silver carp) in Behera Province were recorded (Table 2). The overall infestation rate was rate 87.3%. It was found that *Oreochromis spp.* was the most susceptible species to parasitic infestation (99%) followed by silver carp (97%) and *C. lazera* (66%). The peak of infestation was recorded during winter (98%) followed by autumn (87.3%), spring (82.7%) and summer (81.3%). The recorded ectoparasites were *Trichodina* spp*., Chilodonella hexastica, Apiosoma* spp*., Ambiphrya* spp*., Henneguya branchialis, Myxobolus* spp*.,* and monogenetic trematodes[32]. Black carp *Mylopharyngodon piscens* (152) and common carp (400) were also collected from Abbassa fish farm, Sharkia, to study the prevailing ecto- and endoparasitic diseases. Protozoa (*Trichodina* sp.) affected common carp with total prevalence 65.25%. Seasonal prevalence patterns were as follows: spring 80%, summer 50%, autumn 72% and winter 59%*.* Monogenetic trematodes infected common carp with an

overall prevalence of 56.5%. Seasonal prevalence was spring 30%, summer 73%, autumn 69%, and winter 54%. Encysted metacercaria of *Centrocestus formosinus* were isolated from black carp, with total prevalence 100% throughout the year. Encysted *Diplostomum* sp. metacircaria were isolated from common carp with total prevalence 0.5%. In terms of nematodes, *Capillaria* sp. was isolated from the intestines of black carp and common carp with overall prevalence values of 56% and 30.75%, respectively. Seasonally, the prevalence of *Capillaria* among infected black carp was spring 61.4%, summer 77.4%, autumn 30%, and winter 9.1%, while for common carp prevalence during spring was 44%, summer 48%, autumn 7% and winter 24%. The nematode *Paracamallanus cyathopharynx* was also isolated from the intestine of black carp by total prevalence of 7.93% and maximum seasonal prevalence during spring of 21%. The parasite was not recorded during summer, autumn or winter. The crustacean *Lernaea cyprinecea* was recorded in common carp at an overall prevalence of 22.5%, with seasonal prevalence of spring 2%, summer 74%, autumn 14% and winter not recorded. Leeches were recorded in 1.5% of common carp and a prevalence during spring of 6%, and a complete absence during the other seasons[33].

During 2008, a *Cleidodiscus aculeatus* infection was seen and associated with mass mortalities of *Cyprinus carpio* reared in tanks at the Abbassa Fish Farm (Table 2). All dead fish had high parasite abundance (mean abundance [± S.D.] = 148.3±22.5), entangled in the gills. Fish (73.2%) harbored the parasite with intensities ranging between 5 and 12 parasites per fish[34].

During 2009, The prevalence of isolated Protozoa from *Oreochromis niloticus* fingerlings collected from a cultured fish farm in Giza showed high infestation rates with *Trichodina mutabilis* (71.3%), *Chilodonella hexasticha* (60%). Monogenetic flukes (*Gyrodactylus* rysavyi) had infestation rate of 40%, while digenetic larvae (Hetrophyid metacercariae) showed an infestation rate of 66.6%. Also the prevalence and intensity of infection by *Lernaea cyprinacea* among three carp species were detected. A total of 450 fish were examined. The overall prevalence of infestations by *Lernaea cyprinacea* was 50.4%. Silver carp has the highest prevalence of *Lernaea cyprinacea* (62.7%), followed by grass carp (49.3%), then mirror carp (39.3%)[35].

During 2010 the metazoan parasitic infestation of African catfish, *Clarias garipienus* collected from January to December 2010 from Al-Manzala fish farm; Dakahlyia Governorate. Nine hundred and eighty four parasites were collected from 344 fish samples out of 500 African catfish (*Clarias garipienus*); different parasitic genera, trematodes (monogenetic *Quadriacanthus clariadis* and digenetic *Orientocreadium* sp.), cestodes (*Polyonchobothrium* sp.) and unidentified encysted metacercariae

(EMC) were recovered. Parasites were collected from different body parts of the fish. Prevalence, intensity and abundance of the infection with parasites varied with season. Several histopathological changes were observed in fish organs; gills, accessory respiratory organ, skin, musculature, heart, anterior and posterior kidneys, liver, spleen, and intestine[36].

During 2012 the prevalence of *Anguillicolacrassus crassus* infection in the European eel *Anguilla anguilla* collected from Alexandria, Sharkia and Dakahlia fish farm, was 63%, with 4.49 mean parasite intensity per infected fish. The highest infection rates were recorded in spring and winter (79.3 and 70%), respectively. The lowest infection rates were recorded in autumn and summer (53.3 and 49.3%), respectively[37].

2.3 Mycotic Infections

Fungi are responsible for a number of economically important diseases in teleosts. They cannot use photosynthetic pathways for energy production as they have no chloroplasts and therefore must live a saprophytic or parasitic existence. The Oomycetes (*Saprolegnia*, *Achyla*, *Branchomyces*) group is the most important of the fungal pathogens and are commonly seen during winter and are associated with stress factors. They are widely distributed in aquatic habitat and very few are parasitic. Oomycetes have a common characteristic feature of producing motile biflagellate spores that can cause infection to occur at any time. Saprolegniasis is a common and highly prevalent fungal disease that affects all species and ages of freshwater and estuarine fish. Several factors are involved in the development of fungal infections in fish. These factors may affect the fish or the fungus and it is a combination of factors rather than any single condition which ultimately leads to infection. It has long been considered that the fungi responsible for saprolegniasis are secondary pathogens, and lesions are commonly seen after handling and after traumatic damage to the skin, in overcrowded conditions and in conjunction with pollution or bacterial or parasitic or viral infections. Temperature has a significant effect on the development of infections. Most epizootics occur when temperatures are below the optimal temperature range for the species of fish. As the majority of fungal infections are secondary invaders, the review of fungal infection is included in the section on mixed infections.

2.4 Viral infections

Viruses cause clinical or subclinical problems with negative impacts on the economy of fish production. Although members of twelve virus families have been identified

in wild and cultured fish worldwide, there is currently little information about viruses infecting fish populations in Egypt. Only three records indicate the presence of infectious pancreatic necrosis virus (IPN) and spring viremia virus (SVV) among freshwater fishes[38-40]. The knowledge gap can be filled using a discovery-oriented fish research system. Based on multidisciplinary collaborative activity and utilizing molecular markers and molecular biology technology, such a system could give a comprehensive picture of the current status of fish viruses in Egypt within a few years.

2.5 Infectious diseases in hatcheries

During 2000 a *Saprolegnia diclina* infection was observed during winter among Nile tilapia hatcheries in Sharkia Province. Mixed bacterial (54%) and parasitic (6%) infections were recorded (Table 3). The recovered bacterial isolates were identified as *Flexibacter columnaris* (8%), *Aeromonas hydrophila* (8%), *Pseudomonas fluorescens* (12%), and mixed infection of *A. hydrophila* and *P. fluorescens* (14%). The detected ecto-parasites were *Trichodina* sp. (2%) and *Lamproglena* sp. (4%). Single infection by *Saprolegnia diclina* was prevalent (40%)[41].

During 2001 aeromonads and pseudomonads together with *Ichthyophthirius multifiliis* and *Dactylogyrus spp*. were obtained from *Oreochromis niloticus* reared in hatcheries in Aswan Governorate (Table 3). *Aeromonas hydrophila* was the highest virulent strain, causing 100% mortalities within 5 days of infection while *Pseudomonas fluorescens* infection caused 60% mortalities within 8 days[42].

During 2002 mortalities due to *Aeromonas hydrophila* and *Flexibacter columnaris* as well as *P. fluorescens* were recorded at EI Mahzala, Nawa, EI-Tal EI-Kebeer and Abbassa fish hatcheries (Table 3). That same year, lernaeosis was recorded among common carp, grass carp, silver carp, black carp and Nile tilapia from the fish hatchery of the government's Central Laboratory of Aquaculture Research (CLAR), Abbassa, with an overall prevalence of 20.76[43].

During 2004 Beni-Souef hatchery was visually inspected for parasitic lernaeids from brood and grow-out stocks (Table 3). The prevalence of the lernaeosis among broodstock of silver carp, grass carp and common carp were 38.8%, 39.6% and 39.4%; respectively. By contrast, prevalence among small sized carps of the same species was 39.6%, 61.7% and 54% [44].

Table 3. Pathogens recorded from freshwater Egyptian fish hatcheries.

Year of record	Type of Infection	Species affected	Site
2000	*Saprolegnia diclina, Flexibacter columnaris*, *Aeromonas hydrophila*, *Pseudomonas fluorescens, Trichodina* sp.*, Lamproglena* sp.	Nile tilapia	Sharkia
2001	*Ichthyophthirius multifiliis, Dactylogyrus* spp., *A. hydrophila, P. fluorescens*	Nile tilapia	Aswan
2002	*A. hydrophila, F. columnaris*, *P. fluorescens,* lemaeosis	Nile tilapia	EIMahzala, EITal-EIKebeer Abbassa
2004	*L. cyprinacea*	Grass carp, silver carp and common carp	Beni-Suef
2009	*P. aeruginosa, P. fluorescens, L. cyprinacea*	Nile tilapia, African catfish, common carp, grass capr, silver carp	Behera, Domiata, Abbassa

During 2009, *Pseudomonas aeruginosa* and *P. fluorescens* (Biovar I, II, III, IV, and V) were isolated from silver carp broodstock, which exhibited 65% mortality following their transfer from Behera Province to Domiata Province (Table 3). The microorganisms were highly virulent to all tested cyprinids, moderately virulent to Nile tilapia and African catfish and virulent to mugilids[(45)]. During the same year, the crustacean parasites, especially *Lernaea* spp., were reported to cause serious economic problems and high mortality rates among fish hosts in carp hatcheries in the CLAR hatchery, Abbassa. The overall prevalence of infestations by *L. cyprinacea* was 50.4%. Silver carp had the highest prevalence (62.7%), followed by grass carp (49.3%), then mirror carp (39.3%). Among immature fish, the prevalence was higher in silver carp (72%) than in grass carp (54%) or mirror carp (45%). Also, among mature fish, the incidence was higher in silver carp (44%) than in grass carp (40%), or mirror carp (28%). Among immature fish, the intensity of infestation (i.e. counts per fish) was highest in silver carp (3-53), followed by mirror carp (4-28), then grass carp (4-22). Among mature fish, intensity was highest in silver carp (6-60); followed by grass carp (4-30) and mirror carp (10-20)**[(46)].**

3. PREVENTION AND CONTROL OF FISH DISEASES

Infectious disease occurs when a virulent pathogen, obligate or facultative, is able to overwhelm the defense mechanisms of a susceptible host under environmental conditions that are conducive to the disease process. Prevention is the cornerstone of any health protection program and can be as challenging and complex as the actual control of existing diseases. The control of fish diseases includes both preventive and treatment measures.

The key elements of disease prevention include:

- Knowledge of pathogen transmission.
- Reliable detection of disease carriers.
- Development of effective methods to limit the entry of pathogens or carriers into fish cultural facilities.
- The capacity to provide environmental conditions conducive to good fish health.

3.1. Prevention of fish disease

Regulatory and Cooperative Measures

Avoidance of disease is a fundamental part of programs developed to protect the health of man and domestic animals. Regulatory and cooperative measures can be effective in preventing exposure to physical, chemical and biological disease agents. Regulations should be developed and applied to provide organizational structure and to assure the execution of procedures to contain diseases and their pathogens and to guide the action to be taken when outbreaks occur.

Regulations for fish health protection are most useful in the control of those diseases clearly identified as being caused by obligate fish pathogens. It is essential to have the capability to accurately and timely diagnose these diseases and to have both governmental and industry support behind any effort to develop and implement regulations. Properly designed and applied regulatory programs can help solve certain problems that cannot be effectively dealt with by other less restrictive methods. There are many other important elements of fish health management that should be considered before regulation, as discussed below.

Facilities, Water Supplies, and Environmental Manipulation

Disease prevention in fish culture is, to a large degree, a function of the nature of a facility and how it is managed. Successful fish culture is largely the result of effective environmental manipulation (design of the facility and the nature of its water supply). The occurrence of infectious disease is often related closely to

environmental stress. Environmental conditions imposed on fish are determined by site selection, water supply characteristics, facility design, fish handling and transport systems, and the efficiency of waste removal.

Nutrition and Feeding

Proper feeding of a nutritious diet is important, not only for growth and prevention of nutritional deficiencies, but also for the overall health and vigor needed to cope with a variety of disease agents. Fish under intensive culture rely entirely upon the nutritive quality of artificial feeds. Diet selection, feeding frequency, and quantities fed are controlled by the fish culturist. Nutritional problems, arising from dietary imbalances, continue to cause problems in cultured fish even though great advances have been made in the knowledge of the nutrient needs of fish. There is strong evidence in the literature on the role of nutrition in disease resistance[(47)].

Genetic Resistance to Disease

The concept of genetically enhancing the resistance of fish to disease has intrigued workers for many years[(48)]. The loss of genetic diversity, as often happens in hatchery management, makes it difficult to develop strains of fish that are resistant to several diseases at once. Generally, by maintaining a high level of genetic diversity in a stock and by developing hybrid vigor, there should be potential for breeding fish strains with an enhanced ability to withstand stress and infectious disease agents. The process of selecting strains of fish that are resistant to a specific disease can create another problem. Disease-carrying populations of fish have been maintained at some installations to allow for “natural selection” in survivors and as a practical method of challenging selected stocks to measure any increases in resistance. Fish strains to be tested were held in water that already had passed through an infected population.

Vaccination

Rapid progress has been made in research on the immune responses of fish and in the development of immunization procedures[(49)]. Vaccines do not provide absolute protection from infection but do help fish combat infections sufficiently to make immunization cost-effective in many situations where specific diseases cause repeat problems. As a result, licensed vaccines are now available against vibriosis, enteric redmouth, and furunculosis diseases. The development of vaccines against Egyptian pathogens in a national vaccination center in strongly recommended.

Sanitation and Disinfection

The goal of a sanitation program is to prevent the transfer of fish pathogens from one place to another. Little information has been published regarding the methodology for ensuring sanitation of fish culture facilities, disinfection procedures,

or the evaluation of cost-effectiveness of different sanitation measures[50]. Egg disinfection strives to prevent the vertical transmission of pathogens from the parent stock to the progeny and to prevent horizontal transmission from the egg facility to the rearing facility. During the rearing of fish, sanitation measures can be helpful in maintaining different stocks of fish in isolation from one another.

Disinfection can be carried out using a phased approach or in a single, facility-wide operation. Phased disinfections can be performed whenever a facility cannot be de-populated and disinfected in a single operation. Total facility disinfection disrupts fish production, but is easier to carry out. There is also a better chance of success in total facility disinfection than in a phased operation because the risk of recontamination is reduced[50].

3.2. Disease control methods

The objectives of control measures for infectious diseases are to:

- Reduce or eliminate the source of infection.
- Break the connection between the source of infection and susceptibility of fish.
- Reduce the susceptibility of fish to infection.

Practical guidelines on how to control infectious diseases are provided in Annex 1.

Reducing or eliminating sources of infection

- Accurate disease diagnostic techniques and sensitive pathogen detection methods are essential.
- Method of disease spread from fish to fish and from place to place must be determined.
- Steps can be taken to prevent the spread of disease by controlling the transfer of infected fish or eggs into areas believed free of disease.
- Elimination of infected carriers from the water supply to a facility and the introduction of specific therapy programs to reduce disease.
- Quarantine is the best method to reduce disease introductions. Introduction of exotic fish provides a degree of both benefit and risk.

The risks include the possible introduction and establishment of a disease. If a disease is suspected but not clearly established, it is best to consider both precautionary and control methods. Details of aquatic animal quarantine are given in Annex 2.

Breaking the connection between the source of infection and susceptible fish

This step can be initiated as soon as research findings indicate which methods might be effective, even though significant sources of infection still exist. Examples of measures include:

- Broodstock populations which carry disease agents should be treated or eliminated.
- Stream water supplies may harbor infected carriers but the connection between the sources of infection and the cultured fish can be broken through the use of water sterilization equipment.
- Pasteurization of feed and feed ingredients can be used to break the link between source of infection and susceptible fish.
- Disinfection of rearing facilities between stocking of fish year-classes can also help break the connection between an infected stock and the next group of fish to be reared.

Reducing the susceptibility of fish to disease

- This can be achieved not only by addressing endogenous factors, such as species and strain of fish, immunocompetence and age, but also by improving fish's ability to adjust physiologically to changes in the external environment.
- Adjusting environmental conditions to reduce adverse effects. Methods should be sought to regulate water temperatures, alter oxygen and other dissolved gas levels, reduce ammonia and nitrite levels, reduce population densities, and to improve handling methods to protect the integrity of the skin, scales and mucous membranes of fish.
- Consider the use of immunostimulants to improve disease resistance (see Annex 3).

3.3 Disease treatment methods

Successful disease control involves a careful program of fish health management that removes infected stocks, prevents re-infection, reduces stress, and maintains optimal production conditions. Unless an effective fish health management program is promptly initiated, disease will reoccur whenever stresses that increase susceptibility reappear. If fish are provided with a good environment and adequate nutrition, the risk of infection by pathogens is greatly reduced.

Chemotherapy

Chemotherapy is defined as the use of drugs and chemicals for the treatment of infectious disease. To be useful, the chemicals must be effective against the pathogen without significant adverse effects on the fish host. The first successful chemical was probably salt, used as a dip treatment to reduce pathogens on external surfaces. Guidelines for chemotherapy are provided in Annex 4.

Antibiotics

Antibiotics are very useful additions to a fish health manager's toolbox, but they are only tools and not "magic bullets". The ability of antibiotics to help eliminate a fish disease depends on a number of factors:

- Does the problem have a bacterial component?
- Are the bacteria involved sensitive to the antibiotic chosen?
- Are the proper dosage and treatment intervals being used?
- Have other contributing stresses been removed or reduced?

Guidance on use of antibiotics is provided in Annex 5.

4. ECONOMICS OF DISEASES CONTROL IN EGYPTIAN AQUACULTURE

Pond farm production accounts for around 85% of the volume of total aquaculture production in Egypt (Table 4). Interviews were carried out by WorldFish staff (unpublished data; 2011-2012) to explore the strategies for fish health management used by fish farmers. Disease outbreaks were reported as a problem in all three governorates (Kafr El Sheikh, Behara and Sharkia). The interviews revealed that of 13 farms in Behera, with an average of 22,000 cultured tilapia per farm, and with a total of 286,000 cultured tilapia (379 feddan[1]), *Saprolegnia* was reported at two farms (average 44,000 tilapia) and *Aeromonas* infection was reported at three farms (average fish holdings 66,000 tilapia) and during the two infection types two treatments were applied (salt treatment for *Saprolegnia* and oxytetracyclin for *Aeromonas*).

Of 14 farms in Sharkia that were investigated, with an average of 15,000 tilapia per farm and with with a sample total of 210,000 cultured tilapia (461 fedan), *Saprolegnia* was detected in two farms (average 30000 tilapia) and during the infection two types of treatments were applied (potassium permanganate and antibiotics).

[1] 1 feddan = approximately 1 acre (0.4 ha).

In Kafr-Elsheikh, of the 34 farms surveyed, with an average of 17,000 tilapia per farm and with a sample total of 578,000 cultured tilapia (1254 fedan), *Saprolegnia* was detected on seven farms (average numbers of fish held = 119,000 tilapia). Two farms were also infected with *Aeromonas*, and during the infection period two treatments were applied (the antifungals Anticide and ciprofloxacin).

Table 4: Data on farmed fish production on sample farms in three governorates [51]***, together with disease prevalence***. **Source:** ***GAFRD (2010), CAPMAS (2011), and authors' calculations.***

Parameter	**Kafr el Sheikh**	**Behera**	**Sharkia**
Numbers of fish ('000s	2875 (4%)	5206 (7%)	5876 (7%)
Area of pond production (feddan)	143,727 (40%)	14,229 (4%)	35,011 (10%)
Total pond fish production(tonnes)	324,479 (55%)	31,292 (5%)	76,845 (13%)
Tilapia production (tonnes)	259,583	23,568	62,176
Mullet production (tones)	14,966	1,553	3,831
Carp production (tonnes)	42,383	4,610	10,838
Catfish production (tonnes)	7,547	n/a	n/a

Notes: Percentage figures in parentheses represent the percentage contribution of fish production in the governorate to total Egyptian fish production. Carp species include common, silver, and bighead.

According to the literature, infection of tilapia during the growing season with either *Aeromonas hydrophila*, *Pseudomonas fluorescens* and/or *Saprolegnia diclina* is associated with 40-90% morbidity (average 70%) and 10 – 50% mortality (average 30%).

Cost scenarios associated with diseases and their treatment are presented in Annex 6.

5. DISCUSSION

Fish has become an important resource in Egypt to meet the food and nutrition security needs of a rapidly expanding human population. Aquaculture and fish farming conditions should be improved in a way that controls the spread of disease, which negatively impacts on the development of the sector. Fish disease is rarely a simple association between pathogen, a host fish and environmental problems, such as poor water quality, and other stressors often contribute to the outbreak of infectious and non-infectious diseases. As can be seen from the above review, bacteria are responsible for many diseases and heavy mortality in cultured fish. Most

of the causative micro-organisms are naturally occurring saprophytes, which utilize the organic and mineral matter in the aquatic environment for their growth and multiplication. Secondly, parasites infect fish far more than any other group of pathogenic organisms. There are both opportunistic parasite pathogens and also a number of obligate parasites that kill the host or interfere with growth and reproduction. Some are also of zoonotic and public health importance.

Because of the lack of legislation and poor public service veterinary services, it is recommended that hatcheries and producers produce their own plans for early identification and control of key fish diseases.

The production of larvae and fry remains risky for some species because of the lack of control of the microbiota in rearing systems. Conventional approaches, such as the use of disinfectants and antimicrobial drugs, have had limited success in the prevention or cure of aquatic animal disease. Use of antibiotics is also inappropriate because it can result in an imbalance of microflora for the fish larvae and promote antibiotic resistance. The development of a disease control program is a better and cheaper approach to disease prevention and control, especially in hatcheries.

Immunostimulants offer one alternative strategy to the use of antimicrobials in disease control and have already been widely developed and successfully applied in aquaculture.

As aquaculture practice in Egypt is developing and becomes increasingly complex, conflicts with other resource users will increase. There are also growing environmental concerns as farming practices intensify. The potential conflicts and concerns require careful evaluation and proper management. The Egyptian Ministry of Water Resources and Irrigation (MWRI), Ministry of Agriculture and Land Reclamation (MOALR), as well as the Ministry of Environment (MOE) must take the lead in tackling this important issue. The government of Egypt should increase their support to the aquaculture sector as a source of animal protein, while paying close and careful attention to aquatic environmental quality.

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ANNEX 1: PRACTICAL MEASURES TO REDUCE OR ELIMINATE SOURCES OF INFECTION

- Accurate disease diagnostic techniques and sensitive pathogen detection methods are essential;
- Method of disease spread from fish to fish and from place to place must be determined;
- Steps can be taken to prevent the spread of disease by controlling the transfer of infected fish or eggs into areas believed free of disease;
- Elimination of infected carriers from the water supply to a facility and the introduction of specific therapy programs to reduce disease;
- Quarantine measures have been useful in containing outbreaks of disease in new areas after a disease control program has been put into operation.

Farmers should be aware of general signs of fish diseases:

- The presence of dead or dying fish.
- Fish often stop feeding and may appear lethargic.
- Healthy fish should eat aggressively if fed at regularly scheduled times.
- Pond fish should not be visible, except at feeding times.
- Fish are observed moving listlessly in shallow water,
- Fish are gasping at the surface, or rubbing against objects.
- Other behavioral abnormalities.
- Physical signs include the presence of sores (ulcers or hemorrhages), ragged fins or abnormal body confirmation (e.g. a distended abdomen or "dropsy" and exopthalmia or "popeye").

Veterinarians should follow the guidelines required to accurately diagnose fish diseases, summarized as:

- Case history, dates of fish stocking, size of fish at stocking, source of fish, feeding rate, growth rate, daily mortality and water quality.
- Clinical signs, good records of behavioral and physical signs exhibited by sick fish, as well as morbidity and mortality rates.
- Check the water quality, especially dissolved oxygen, ammonia, nitrite, and pH, total alkalinity, total hardness, nitrate (saltwater systems) and chlorine (if using city water). Ideally, daily records should be available for immediate reference.
- Postmortem examinations of sick fish.
- Laboratory examinations after very careful sampling.

ANNEX 2: QUARANTINE

Introduction

Quarantine is the best method to reduce disease introductions. Introduction of exotic fish provides a degree of both benefit and risk. The risks include the possible introduction and establishment of a disease. If a disease is suspected but not clearly established, it is best to consider both precautionary and control methods:

- Quarantine reduces the disease potential by the isolation of hosts (however, there is a great difference between disease and pathogen presence);
- The disease agent is not allowed to pass unchecked into a culture system, where it could rapidly increase in numbers;
- If newly arrived stock is placed in quarantine, a disease may be recognized after a suitable incubation period;
- Quarantine may establish a “disease free” or “pathogen free” status of imports.

The purpose of quarantine is to:

- Allow fish to acclimatize to captivity in a controlled environment;
- Allow treatment of disease in a controlled environment;
- Reduce the stress of acclimation;
- Reduce cost associated with medication and fish mortality;
- Allow easy observation of new fish in case of disease.

The development of quarantine measures:

- Facilitates holding and observation of fish in a biosecure environment;
- Allows testing of fish for infectious agents in a diagnostic lab;
- Facilitates access to more specialized laboratories and resources;
- Protects the surrounding aquatic environment and biota;
- Facilitates subdivision of risks into lower and higher categories.

General design principles and security measures that must be implemented during quarantine

- Quarantine facilities should be located within or close to existing fish heath facilities.
- Facilities should have 24 h supervision.
- Facilities should be lockable and access restricted to designated personnel.
- Construction should avoid accidental spill or discharge of water or animals or equipment to the surrounding water.
- Intake water should be obtained from a clean, unpolluted source to prevent physiological stress or masking of infectious agents by opportunistic infections (water analysis recommended).
- No loss or release of quarantined fish.
- No loss of contaminated water or equipment.
- Tanks, ponds, pools or other containers should be isolated from the aquaculture facilities as well as municipal and open water.

- All water leaving quarantine should be considered as potentially infected. It should be discharged into reservoir or pond that permits chemical disinfection or discharge into a land-based pit or pond.
- All equipments used in the quarantine (such as nets, containers, pipes, hoses, pumps) should remain within the containment facility and not be removed or used for any other purpose unless disinfected.

Fish disease laboratory facilities in quarantine facilities:

- Should be located in an enclosed area.
- Should have the materials necessary to prepare samples.
- Should be able to conduct microscopic examinations during quarantine.
- The containers and reagents as well as stains should be available to permit sample dispatch to the diagnostic lab.
- Samples leaving a high–risk quarantine facility should be transported by approved quarantine personnel and be preserved and secured for handling by non quarantine personnel.

ANNEX 3: USE OF IMMUNOSTIMULANTS

Introduction

An immunostimulant is a chemical, drug, stressor, or action that enhances the innate or non-specific immune response by interacting directly with cells of the system, thereby activating them. Innate defense includes both humoral and cellular defense mechanisms, such as the complement system and the processes played by granulocytes and macrophages. Immunostimulants increase immunocompetency by increasing resistance to infectious disease, not by enhancing specific immune responses but by enhancing non-specific defense mechanisms. No memory component is involved and the response is likely to be of short duration. Injection of immunostimulants enhances the function of leucocytes and protection against pathogens. However, this method is labor intensive, relatively time-consuming and becomes impractical when fishes weigh less than 15 g. Oral administration or immersion should thus be used. However, fish cannot be protected against all infectious diseases by immunostimulants.

Immunomodulation of larval fish has also been proposed as a potential method to improve larval survival by increasing the innate responses of the developing animals until their adaptive immune response is sufficiently developed to mount an effective response to the pathogen. The delivery of immunostimulants as a dietary supplement to larval fish may thus be of considerable benefit in boosting innate defenses, with little detriment to the developing animal. During 2004-2009, the senior researcher and program leader of fish health at Worldfish (Dr. Salah Aly) carried out a series of experimental studies on the effect of immunostimulants on growth, survival and disease resistance in Nile tilapia, the most common freshwater fish in Egyptian aquaculture. All the results have been published and their Abstracts are accessible on the internet[52-71].

Factors to be considered in the implementation of immunostimulation strategy:

- Stimulation of an immune system can be too intense and can harm or even kill the host.
- The mode of action of different immunostimulants should be understood.
- The immune system of larvae is poorly developed, consisting mainly of nonspecific defenses.
- The maternal immune defenses are significant only during early developmental stages.
- Research aimed at developing methods for immunostimulation of larvae should prioritize the stimulation of non-specific defense mechanisms, including that of non-specific maternal defenses.

ANNEX 4: CHEMOTHERAPY

Guidelines for use of chemotherapy

- The best treatment is good animal husbandry
- Drugs and chemicals are often used to correct errors in management. While this may be used as a stop-gap, it cannot be used to prop up poor culture programs.
- Indiscriminaet use of therapeutic agents should be avoided.
- The continuous feeding of low levels of antibiotics in the diet as a prophylactic measure against outbreaks of bacterial disease during periods of stress, or to improve growth rates, are questionable practices. It results in the removal of only those bacteria most sensitive to the drug and can lead to the development of drug resistant strains. Drug resistant bacteria can transmit resistance to bacteria that have never been exposed to the drug.
- Treatment with antibiotics is recommended only when needed, and then only at prescribed treatment levels.
- If it is decided to use antibiotics, treatment should be conducted for the full time period required. Foreshortened treatments encourage the development of drug resistance and can lead to the need for elevated drug levels, and eventually, to loss of effectiveness.
- The casual use of therapeutics on a routine basis is not without possible adverse effects on the general health of the fish and is not recommended,
- Whenever possible, seek a positive diagnosis of any disease problem by a professional fish health specialist.
- Start treatment with the correct drug at the recommended level.
- If a chemotherapeutant is needed, treat quickly and effectively.
- Users are advised to proceed with caution and to follow label directions.
- Recommended rates of treatment are based on the levels that researches have found to be necessary and that various fishes will tolerate.
- Although there is a built-in safety factor, using more than the recommended rate is not necessary, may be harmful, and even illegal.
- A two week withdrawal period from all chemotherapeutic treatments before the intended release or harvest date is recommended.

Guidelines for chemotherapy application

<u>Before treatment</u>

- Ensure that information on chemical characteristics of the water supply is available before application.
- Ascertain how environmental conditions on the farm are likely to affect the toxicity and efficacy of the treatment.
- What will work at one place may not be effective elsewhere because of differences in water chemistry.
- Before using any chemical, be sure to test it first on a small number of sick fish.
- Keep in mind that healthy fish can tolerate chemical treatment more readily than sick fish and that treatment levels may need to be reduced if the fish are weak or in poor condition.

- Ensure that, rearing facilities are clean before treatment. Dirty raceways or tanks may contain organic matter that can absorb part of the treatment chemical and reduce its effectiveness.
- If the fish density is excessive it should be reduced, if possible, prior to static treatment. Supplemental aeration should be provided if needed.
- During hot weather, treatments should be made during the coolest part of the day, using chemicals that create the least environmental hazard or stress.
- Starving fish for l-2 days prior to treatment will reduce oxygen consumption and ammonia production and will increase resistance to scale loss. Treatment within 4 h of feeding should be avoided.
- Any parasitism of the gills should be treated first since such parasites may affect the respiratory capability of the fish.
- Monitor dissolved oxygen levels before treatment. Fish are stressed during treatment and their oxygen requirements increase.
- Before treating with a new compound or formulation or using a product for the first time on an installation, always treat a small group of fish first and watch for unexpected mortality.

<u>During treatment</u>

- Always observe fish during treatment to watch for signs of stress or unexpected toxicity.
- Monitor dissolved oxygen levels during treatment. Fish undergoing treatment will be stressed and their need for oxygen increases.
- Always check calculations (0.1X will be ineffective; 1.0 is effective; but 10X will be fatal). If possible, have the figures corroborated independently.

<u>After treatment</u>

- Keep records of all treatments, their purpose, and the results for future reference.

Methods for chemotherapy application

<u>Treatment in the diet</u>

Commercial feed with antibiotic additives, if available, is cheap and easy to use. Medicated feed stores well and can be used in place of the regular diet. If commercially medicated feed is not available, medicated feed can be prepared on site. It is best to suspend such drugs in oil when preparing medicated feed (cod liver oil seems to have better palatability than soy bean or corn oils, but any of these will do). Once treatment has begun , the recommended dose and treatment schedule should be adhered to. It is a mistake to ry to save money by stopping treatment when mortalities stop, by using less than the recommended amounts, or by reducing the period of treatment.

<u>Localized application</u>

External: Localized skin applications are feasible only for broodstock and other valuable fish. The drug or chemotherapeutant used should be relatively insoluble in water, act on contact, and either be denser than water or readily adhere to the fish.

Internal: For small numbers of valuable fish, injections of antibiotics may be used, but can be prohibitively expensive and labor intensive. Intraperitoneal injection is superior to subcutaneous or intramuscular injection. It may be best to anesthetize the fish with MS-222

(tricaine methanesulfonate), benzocain, clove oil, or some other recommended fish anaesthetic prior to injection.

Bath treatment

*Dip bath:*This involves a short bath treatment varying in duration from a few seconds to 5 min, depending on the chemical and concentration used. Dip treatments are often used on broodstock. While effective, they can be highly stressful. After treatment, fish should be rinsed in clean water before being returned to the holding facility to avoid transfer of chemical to the tank.

*Short baths:*For treatments of <1 h, when fish are held in facilities where fresh water is available and adequate oxygen levels can be maintained, short baths are useful because high concentrations of chemicals can be used. Considerable care is required to avoid chemical overdoses or overly long contact times.

Indefinite treatment

This method is suitable only for treating stock held in fish ponds. Low concentrations of chemical are used and allowed to dissipate in the pond. Treatments may have adverse effects on the biota or on dissolved oxygen levels.

Flush treatments

In treatments of this type, a measured amount of concentrated chemical is added at the inlet and allowed to flush through a pond or raceway. Amounts of chemical used must be accurately determined. Lowering the water level in the holding unit reduces the quantities of chemical needed and also facilitates rapid dilution of the treatment when fresh water is added to restore normal conditions.

Constant flow

In constant flow treatments, the chemical is metered into the water inflow at a constant rate to maintain a given concentration for a given period of time. This method requires accuracy and is expensive in terms of the amount of chemical needed. The method requires no special attention to oxygen or ammonia levels, since the water flow remains unchanged.

ANNEX 5: USE OF ANTIBIOTICS

General considerations before using antibiotics

- Antibiotics only control the population of bacteria in a fish long enough for its immune system to eliminate them.
- Before antibiotics are even considered, sources of stress such as poor water quality (including sudden and large temperature changes), nutrition, genetics, and handling or transport must be removed or reduced.
- Affected fish should also be examined for parasites.
- Any of the above factors – and, indeed, others, such as attacks by predators - may be the primary cause of disease, as bacterial infections are often secondary to such management problems.
- Contacting a fish health specialist early in a disease outbreak helps identify contributing stresses and the rate of bacterial infection, thereby reducing losses.

Optimal approach to fighting bacterial infections

The ideal solution to bacterial diseases involves working with a fish health specialist to culture the organism and to run sensitivity tests. Although culture and sensitivity tests generally take two or three days, they are, by far, the best methods for selecting an antibiotic that will successfully and economically treat an infection. A fish health specialist should provide instructions on submitting samples to a diagnostic laboratory:
Affected fish should not be treated with antibiotics until after a pathogen sample has been analyzed.

- Samples should be taken from at least 3 to 5 fish showing typical symptoms of the disease.
- Fish that are submitted after they have been given antibiotics often provide poor culture results.
- While waiting for the culture results, the fish health specialist may suggest a broad-spectrum antibiotic that can be used until culture and sensitivity tests have been completed.
- Legalities must also be considered when selecting antibiotics.
- Fish health specialists will be able to provide information on legal constraints for specific antibiotics, information on appropriate dosages, methods of administration and other concerns.

Proper dosages and treatment regimes

Although selecting the correct antibiotic is an important first step in controlling bacterial disease, proper administration of any antibiotic for the recommended number of days is equally important.

- Fish health specialists should provide instructions on the amount of antibiotic to use (dose), the frequency and duration of treatment.
- Withdrawal time (time required, after the last dose of antibiotic has been given, till selling the fish) should be known.
- The pharmacokinetics of a specific antibiotic should be determined.

Consequences of improper dosage and treatment time

If the dose is too high or treatment times are too long, there is a danger of toxicity to the fish, frequently causing liver, kidney, or other organ damage that may or may not be reversible.

If the dose of antibiotic is too low or treatment time is too short, the bacteria will not be killed or weakened sufficiently for the immune system of the fish to remove them, greatly increasing the risk of the bacteria developing resistance to the antibiotic.

It is important to remember that fish diseases are not the only constraint facing freshwater aquaculture in Egypt: many other environmental factors and poor management practices also contribute to low productivity. Water quantity, extreme or changeable temperatures, and the quality and quantity of feed used, all increase the risks of disease. Knowing how to minimize the risks of disease outbreaks, how to monitor for key risk factors and what to do when they occur, can make the difference between a farm being profitable and not. Low temperatures during the winter season predispose farmed fish, especially tilapia, to attack by bacterial and fungal pathogens *Aeromonas hydrophila*, *Pseudomonas fluorescens* and *Saprolegnia diclina* are particularly prevalent and can cause massive mortalities. However, a number of studies suggest potential application of immunostimulants and probiotics in improving fish health and increase resistance to infections, one month of application providing protection for 1-2 months (see papers published by Dr. Salah Aly in this regard[42-61]).

ANNEX 6: COSTS OF DISEASE TREATMENT

To estimate the cost of disease-associated losses and the cost of control measures, we assume a farm or hatchery of 100,000 tilapia to be used as standard. We consider infections by *Aeromonas hydrophila* or *Pseudomonas fluorescens* and/or *Saprolegnia diclina* and their control in any Egyptian aquaculture or hatchery during the current year.

I. Young tilapia

The cost of an infection

To calculate the cost of disease-associated losses, let us consider the example of a farm culturing 100,000 young tilapia (average body weight = 75 g).

1. *Cost of losses due to mortality*:
Mortality = 100000 tilapia x 30/100 mortality =30,000 tilapia
Weight loss = 30,000 tilapia x 75 g (*average body weight*) = 2.250 tone
Economic Loss = 2.250 t x 6000 LE (*price/t*) = **13,500.00 LE**

2. *Economic cost of antibiotic treatment:*
Total BW= 100,000 tilapia X 75 g (average body weight) = 7500 kg
Quantity of antibiotics = 37.5 g (*5 g antibiotic /1000 kg BW/day*) X 7 days = 262.5 g
Economic costs = 262.5 g (antibiotic) X 5 LE (*price/g antibiotics*) = **1312.5 LE**

3. *Cost of losses due to stunted growth during the disease course*:
Assume 30% mortality, 700,000 tilapia remained (feeding rate @ 3% body weight/day)
The amount of feed/day 700,000 X 75 g = 52.5 t X 3% = 157.5 kg
The amount of feed /month = 157.5 kg x 30 day (*no growth*) = 4.725 ton (*of no value*)
Losses due to stunted growth = 4.752 /1.7 (FCR) = 2.77 t (*loss in growth*)
Economic losses due to stunted growth = 2.77 t x 6000 LE (*price/ton*) = **16,620 LE**

4. Total economic loss due to the infection =
13500 $^{(Mortality)}$ + 1312.5 $^{(Treatment)}$ + 16620 $^{(stunted\ growth)}$ = **31,432.5 LE**

The cost of controlling an infection

To determine the cost of control measures, we will give same example for a hypothetical farm with 100,000 tilapia, of average body weight of 75 g.

The amount of feed / day = 100000 x 75 g (average BW) x 3% (feeding rate) = 225 kg
The amount of feed / month = 225 kg x 30 day = 6.75 t

1. Probiotics (used at a rate of 3 g/kg, with average market price 100 LE/kg)
Amount of probiotics needed to provide 1-2 month protection = 6.75 t x 3 g = 20.25 kg
Cost of probiocs needed for one month application = 20.25 kg x 100 LE = **2025 LE**

2. Immunostimulants (Garlic used at a rate of 40g/kg, at an average market price of 5 LE/kg)
The amount of garlic needed to provide 1-2 month protection = 6.75 t x 40 g = 270 kg
Cost of garlic needed for one month application = 270 kg x 5 LE = **1350 LE**

3. Vaccine
Price for prepared vaccine is unlikely to exceed **1500 LE** and will provide protection for 4-6 months.

II. Adult tilapia:

The cost of an infection

In order to estimate the cost of diseases-associated losses, let us assume a hypothetical farm with 100,000 near market size tilapia, with an average body weight of 250 g.

- *Cost of losses due to mortality*:

Mortality = 100,000 tilapia x 30/100 mortality =30,000 tilapia
Weight loss = 30,000 tilapia x 250 g (*mean body weight*) = 7.5 t
Economic loss = 7.5 t X 9000 LE (*price/t*) = **67,500 LE**

- *Cost of losses due to antibiotic treatment:*

Total biomass = 100,000 tilapia x 250 g (*average body weight*) = 25 t
Amount of antibiotic=125 g (*5 g antibiotics /1000 kg BW/ day*) X 7 days= 875 g
Financial loss = 875 g (*antibiotic*) x 5 LE (*price/g antibiotics*) = **4375 LE**

- *Cost of losses due to stunted growth during disease treatment*:

Assuming 30% mortality, 700,000 tilapia remain (feeding rate 3% body weight per day)
The amount of feed fed/day = 70,000 x 250 g = 17.5 t X 3% = 525 kg
The amount of feed /month 525 kg x 30 day (*no growth*) = 15.75 t (*of no value*)
Losses due to stunted growth = 15.75 t /1.7 (FCR) = 9.26 t
Financial loss due to stunted growth = 9.26 t X 9000 LE (*price/t*) = **83340 LE**

- *Total loss due to the infection*

67,500 $^{(Mortality)}$ + 4375 $^{(Treatment)}$ + 83,340 $^{(stunted\ growth)}$ = **155,215 LE**

The cost of controlling an infection

To determine the cost of control measures, we will give same example of a farm with 100,000 tilapia of average body weight 250 g.

The amount of feed / day = 100,000 x 250 g (average BW) X 3% (feeding rate) = 750 kg
The amount of feed / month = 750 kg x 30 day = 22.5 t

1. Probiotics (used as 3g/kg with average market price 100 LE/kg)

Amount of probiotics required to provide 1-2 month protection = 22.5 t x 3 g = 67.5 kg
Cost of probiocs needed for 1 month application = 67.5 kg x 100 LE = **6750 LE**

2. Immunostimulants (garlic used @ a rate of 40g/kg, at average market price 5 LE/kg)
   The amount of garlic needed to provide 1-2 month protection = 22.5 t X 40 g = 900 kg
   Cost of garlic for 1 month application = 900 kg x 5 LE = **4500 LE**

3. Vaccine

- Price for prepared vaccine does not exceed **2500 LE** and gives protection for 4-6 months.

III. Tilapia Fry

The cost of an infection

In order to estimate the cost of disease-associated losses, assume a hypothetical farm producing 100,000 tilapia fry of average body weight 1 g.

1. Cost of losses due to mortality:
Mortality = 100,000 fry x 30/100 mortality =30,000 fry
Financial loss = 30,000 fry x 100 (*price/1000 fry*) = **3000 LE**

2. Cost of losses due to treatment using antibiotic:
Total BW= 100,000 tilapia X 1 g (average body weight) = 100 kg
Quantity of antibiotic = 500 mg (*5 g antibiotic /1000 kg BW/day*) X 7 days = 3.5 g
Economic cost = 3.5g (antibiotic) X 5 LE (*price/ 1g antibiotics*) = **17.5 LE**

3. Cost of losses due to stunted growth during disease treatment[2]:
Assuming 30% mortality, 700,000 tilapia remain (feeding rate 3% body weight per day)
The amount of feed /day 700,000 x 1 g = 70 kg X 30% = 21 kg
The amount of feed /month = 21 kg x 15 day (*no growth*) = 315 kg (*of no value)*
Losses due to stunted growth = 315 /1.7 (FCR) = 185 kg

The cost of controlling an infection

To determine the cost of control measures, we will give same example for any farm cultured with 100,000 tilapia fry with average body weight 1 g.

[2] N.B. Fry would typically increase in biomass to 185 kg. The average size will be 3.64 g instead of 1 g. The average price will increase from 100/1000 fry (value = 7000 LE) to 250/ 1000 fry for fry of 3.6 g (value = 17500). However, the real loss in this case is that fry which are stunted will not grow and may be susceptible to infection and liable to die if stressed.

The amount of feed /day = 100,000 X 1 gm (average BW) X 30% (feeding rate) = 30 kg
The amount of feed /month = 30 kg X 30 day = 900 kg

1. Probiotics (used at a rate of 3g/kg, with average market price 100 LE/kg)
   Amount of probiotics needed to provide 1 month protection = 900 kg x 3 g = 2.7 kg
   Cost of probiocs needed for 1 month protection = 2.7 kg x 100 LE = **270 LE**

2. Immunostimulants (Garlic used aat a rate of 40g/kg with average market price 5 LE/kg)

Amount of garlic needed to give 1- 2 month protection = 900 kg x 40 g = 36 kg
Cost of garlic needed for 1 month application = 36 kg x 5 LE = **180 LE**

3. Vaccine

Price for prepared vaccine does not exceed **500 LE** and gives protection for 4-6 months.