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# OUTBREAK INVESTIGATION OF *Aeromonas hydrophila* IN TILAPIA GROW-OUT FARMS IN MINALIN, PAMPANGA, PHILIPPINES

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## ABSTRACT

The main objective of this study was to investigate the outbreak of *Aeromonas hydrophila* in tilapia grow-out farms in Minalin, Pampanga, Philippines and to determine the possible risk factors in the occurrence of the bacterium. Majority of the collected tilapia samples showed various signs of bacterial infection such as lesion, fin/skin rot, eye opacity and/or abnormal body coloration. The computed attack rate of *A. hydrophila* in the whole municipality was 40.17%. Significant lower attack rate of *A. hydrophila* was recorded in male tilapias (male = 31.76%; female = 62.50%). The temporal pattern of *A. hydrophila* depicts that tilapia were continuously exposed over a long period of time to a common disease-causing factor. The significant risk factors identified for the occurrence of *A. hydrophila* in grow-out tilapia were the source of water (RR = 9.95) and total dependency on feeding (RR = 4.03). The occurrence of bacterial diseases in tilapia is caused by the interplay of various risk factors such as presence of pathogenic organism and source of contamination, and a susceptible host due to stressful environment.

**Keywords: *Aeromonas hydrophila*, tilapia, attack rate, risk factors**

## INTRODUCTION

Nile tilapia (*Oreochromis niloticus* L.) is an important species for freshwater aquaculture, and the improving of its culture and disease-resistance is a major challenge facing fish culturists [1]. In the Philippines, aquaculture becomes popular because on the overture of tilapia [2].

However, the aquaculture success may be impeded by the incidence of diseases [3].

The impact of different bacteria on tilapia industry in the country is well known among the population and health authorities because of their wide geographic distribution and the serious problems they cause [4].

According to Food and Agriculture Organization (FAO, 2004), bacterial infection in fishes is growing fast at present with an approximately 12% annual increase [5]. Diseases in tilapia cause important economic losses in the world`s industry of aquaculture with an approximate of US $150 million annually [6]. Many cases of fish kill caused by different bacterial species were reported in various countries including Philippines [4]. In 1992, a severe disease outbreak threatened the tilapia industry in the country which is attributed to *Aeromonas hydrophila* [7].

Some of the most common genera of bacteria present in pond-cultured tilapia are *Aeromonas*, *Pseudomonas*, *Vibrio*, *Streptococcus*, *Staphylococcus*, *Mycobacterium*, *Edwardsiella* and *Flexibacter* [4]. A huge number of bacteria in the gut of the fish are taken from water sediment. These natural gut bacteria can cause diseases when environmental and cultural conditions become favorable [8]. Fin rot, dark coloration, exophthalmia, corneal opacity, scale loss, hemorrhage, nodular lesions, focal necrosis and granuloma, hyperanemia and loss of appetite are some of the non-fatal effects of these bacteria [4]. Combinations of the above mentioned symptoms of diseases may lead to heavy mortality in farmed fishes [4].

Minalin, Pampanga is located southwest of the capital town of San Fernando. It is subdivided into 15 barangays namely Bulac, Dawe, Lourdes, Maniango, San Francisco de Asisi, San Isidro, San Nicolas (Poblacion), San Pedro, Sta. Catalina, Sta. Maria, Sto. Domingo, Sto. Rosario and Saplad. Aquaculture is one of the sources of livelihood in Minalin. In fact, it is the fourth leading municipality in Pampanga with respect to area of land allotted for tilapia farming (around 1,755 fishpond units with total area of 2,728.69 ha). Thus, this study investigated the outbreak of *Aeromonas hydrophila* in tilapia grow-out farms in Minalin, Pampanga, Philippines and determined the possible risk factors in the occurrence of the bacterium.

## MATERIALS AND METHODS

### Case Definition

Marketable size tilapia (minimum of 100 g) that were grown in earthen ponds in Minalin, Pampanga, Philippines that suffered either one of the following signs of disease such as lesion, fin or skin rot, eye opacity (unclear eye) and body discoloration between November 2013 to March 2014 were considered in the investigation.

**Collection of Fish and Water Samples**

Tilapia samples were collected in 33 tilapia grow-out farms in 14 barangays of Minalin, Pampanga, Philippines. For every farm, three pieces of tilapia were collected and examined for the presence of *A. hydrophila*. The samples were transported live back to the Fish Pathology Laboratory of the College of Fisheries, Central Luzon State University (CF-CLSU), Science City of Muñoz, Nueva Ecija.

On-site analysis of water quality parameters (temperature, dissolved oxygen, pH, salinity and total dissolved solid) was done using YSI multi-parameter equipment. Separate water samples (6 L) were collected for the analysis of alkalinity, total ammonia nitrogen and phosphorous in the laboratory.

**Surveying**

Surveying was conducted to determine the farm practices being implemented in every tilapia grow-out farm.

**Isolation and Identification of *A. hydrophila***

Lateral and vertical incisions were made in the belly portion of the fish to reveal its internal organs. Smears from kidney were aseptically streaked in Aeromonas Selective Agar for the identification of the bacterium. The plates were incubated at 37°C for 18-24 hours.

**Mapping**

Digital mapping was performed using the ArcGIS software with ESRI base maps and downloaded satellite images as references. Mapping of fishponds was done in a farm-level; each farm was represented by a digitized polygon. Computed attack rate (%) of *A. hydrophila* was incorporated in the digital maps.

**Computation of Attack Rate and Relative Risk**

Line listing of generated data on water quality and *A. hydrophila* analysis were opened in EpiInfo for the computation of attack rate and relative risk.

**Statistical Analysis**

Significant differences were analyzed using Independent Sample T-test under the Statistical Package of Predictive Analysis Software (PASW) Statistics Version 18.

**RESULTS AND DISCUSSION**

**Physical Examination**

Presented in Table 1 is the percentage of the tilapia samples collected in grow-out farms in Minalin, Pampanga, Philippines that manifested physical signs of bacterial infection. Around 85%, 53% and 15% of the tilapia samples had the presence of lesion, fin/skin rot, and eye opacity, respectively. Near 45% of the samples showed abnormal body coloration (e.g. whitening, blackening or reddening of a part or the entire body). The presence of two and three physical signs of bacterial disease accounted to 44.79% and 21.88%, respectively. Only 3.13% of the samples had the appearance of the four signs.

**Bacteriological Examination**

The computed attack rate of *A. hydrophila* in the whole municipality was 40.17%. All tilapia samples in Barangay Dawe were positive to *A. hydrophila* (attack rate = 100%) and no *A. hydrophila* was observed in Barangay San Francisco 1$^{st}$, San Nicolas, Sto. Rosario and Saplad. (Table 2).Significant lower attack rate of *A. hydrophila* was recorded in male tilapias (male = 38.03%; female = 52.00%) because of stronger immunity than females. More of the energy in female fish is devoted to reproduction than for immunity [9] (Table 3).

**Temporal Pattern**

The temporal pattern of *A. hydrophila* is presented in Figure 1. The epicurve depicts that tilapia were continuously exposed over a long period of time to a common disease-causing factor.

**Possible and Identified Risk Factors**

Two groups of possible risk factors in the occurrence of *A. hydrophila* were considered, namely farm management practices and quality of the pond water. The computed relative risk (RR) in each considered risk factor is provided in Table 4. Relative risk having value of >1 indicates positive association (the exposed group has higher incidence than the non-exposed group) while RR value of < 1 indicates negative association (the unexposed group has higher incidence).

Under the farm management practices, exposure of the fish to insufficient/incomplete pond preparation (RR = 1.57) and unsafe source of water (RR = 9.95) yielded an RR >1.Total dependency on commercial feeds had an RR of 4.03.Meanwhile under the bracket of water quality, tilapia exposed to high level of phosphorous resulted to RR>1 (RR = 1.12). The only significant risk factors identified for the occurrence of *A. hydrophila* in grow-out tilapia were the source of water and total dependency on feeding.

**Transmission Pathway: Source and Spread**

Provided in Figure 2 is the distribution of *A. hydrophila* infection in tilapia grow-out farms in Minalin, Pampanga, Philippines. Around 67% of the visited tilapia farms were positive to *A. hydrophila*. This bacterium could be transmitted only through direct contact with infected fish, water and/or equipment.

**Table 1: Percentage of tilapia samples collected in grow-out farms in Minalin, Pampanga, Philippines that manifested physical signs of bacterial infection**

| Physical Manifestations of Bacterial Infection | Proportion of the Sampled Tilapia (%) |
|---|---|
| Lesion | 85.42 |
| Fin or skin rot | 53.13 |
| Eye opacity | 14.58 |
| Body discoloration | 44.79 |
| Double combination | 44.79 |
| Triple combination | 21.88 |
| Presence of all | 3.13 |

**Table 2: Attack rate (%) of *A. hydrophila* in tilapia samples across barangay in Minalin, Pampanga, Philippines**

| Barangays | Positive | Negative | Attack Rate (%) |
|---|---|---|---|
| Bulac | 3 | 6 | 33.33 |
| Dawe | 6 | 0 | 100.00 |
| Lourdes | 4 | 5 | 44.44 |
| Maniango | 3 | 6 | 33.33 |
| San Francisco 1st | 0 | 3 | 0.00 |
| San Francisco 2nd | 2 | 1 | 66.67 |
| San Isidro | 4 | 5 | 44.44 |
| San Nicolas | 0 | 6 | 0.00 |
| San Pedro | 6 | 3 | 66.67 |
| Sta. Maria | 7 | 2 | 77.78 |
| Sta. Rita | 5 | 4 | 55.56 |
| Sto. Rosario | 0 | 9 | 0.00 |
| Saplad | 0 | 6 | 0.00 |
| Minalin | 40 | 56 | 41.67 |

**Table 3: Attack rate (%) of *A. hydrophila* in male and female tilapia samples**

| Sex | Positive | Negative | Attack Rate (%) |
|---|---|---|---|
| Male | 27 | 44 | 38.03[b] |
| Female | 13 | 12 | 52.00[a] |

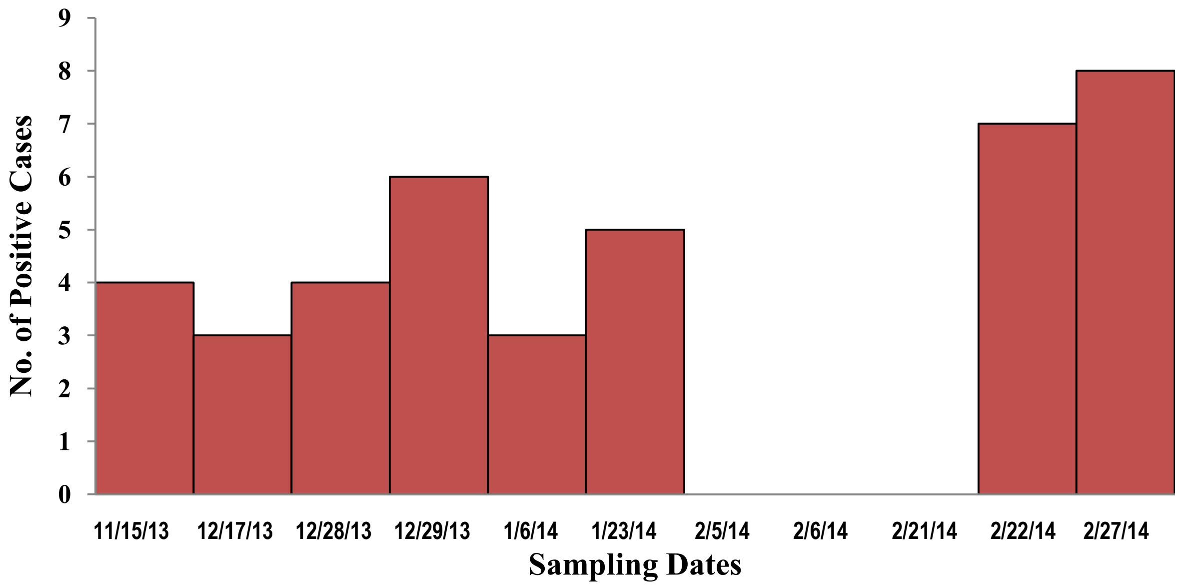


**Figure 1: Occurrence of *A. hydrophila* infection in grow-out tilapia in Minalin, Pampanga, Philippines**

**Table 4: Possible risk factors in the occurrence of *A. hydrophila* infection in grow-out tilapia in Minalin, Pampanga, Philippines**

| Possible Risk Factors | RR Values |
|---|---|
| ***Management Practices*** | |
| **Scale of operation** | **0.76 (0.46-1.23)** |
| **Pond preparation** | **1.57 (0.83-2.95)** |
| **Source of water** | **9.95 (2.56-38.70)*** |
| **Rate of stocking** | **0.67 (0.41-1.01)** |
| **Feeding** | **4.03 (0.63-25.98)*** |
| | |
| ***Water Quality*** | |
| **Temperature** | **0.61 (0.38-0.97)** |
| **Total dissolved solid (TDS)** | **0.80 (0.50-1.28)** |
| **Dissolved oxygen (DO)** | **0.83 (0.48-1.44)** |
| **pH** | **0.48 (0.20-1.20)** |
| **Alkalinity** | **0.64 (0.34-1.20)** |
| **Phosphorous** | **1.12 (0.62-2.05)** |

***significant at p-value <0.05**

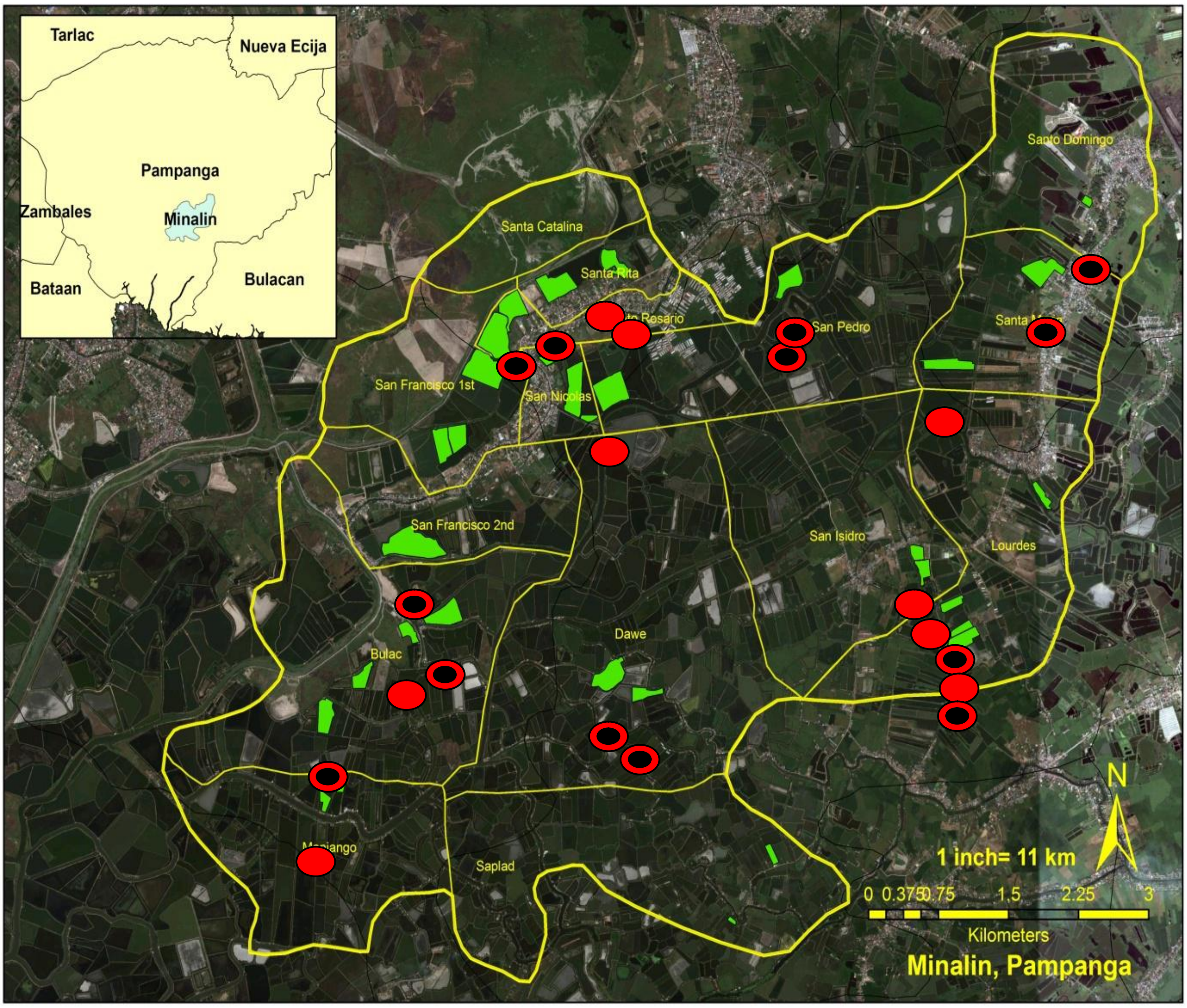


**Figure 2: Distribution of *A. hydrophila* in tilapia grow-out farms in Minalin, Pampanga, Philippines (Note: Red circle = AR <50%; Red circle with black spot = AR >50%).**

Tilapia aquaculture in Pampanga was dependent in Pampanga River, river tributaries and irrigation canals as main sources of water. However, the river was already highly polluted and contaminated based upon physical, chemical and biological analysis of its water and soil [10, 11]. The water had very low DO (2.66 to 3.43 mg/L) and very high phosphorous (0.30 to 0.60 mg/L). High level of phosphorous in water coupled with low DO is good indication of eutrophication [11]. A number of pathogenic bacteria in fishes (*A. hydrophila*, *P.aeruginosa*) and humans (*Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*) were also isolated from the water and soil of Pampanga River [10]. High level of total bacterial count (TBC) and total coliform count (TCC) was observed in the water (TBC = 1.93 x $10^7$ to 2.72 x $10^8$; TCC = 1.45 x $10^5$ to 3.30 x $10^6$) and soil (TBC = 3.62 x $10^9$ to 4.38 x $10^9$; TCC = 1.19 x $10^5$ to 1.06 x $10^6$) [10]

The water source could be considered unsafe to use for the tilapia aquaculture in the province. Basically, the tilapia operators are pumping river water to their pond system which is high in phosphorous, deficient in DO and with the presence pathogenic organisms such as bacteria. Based upon interview, their immediate remedy in case of fish mortality which was water exchange could only aggravate the problem. Contaminated water supply was the main identified risk factor for the occurrence and wide distribution of *A. hydrophila*. This hypothesis was being supported by the type of epicurve that was generated which was common source epidemic (continuous source) (Figure 1). Improper pond preparation practices and intensive stocking could be considered as additional risk factors for the deterioration of the quality of water in ponds. The interviewed farms failed to follow the complete sets of activities involved in pond preparation (draining, elimination of predators and nuisance weeds, harrowing, drying, repairing of dikes, water inlet and outlet canals, screening of water canals, liming, initial filling of water and levelling of pond bottom, and fertilization).Pond preparation should be done properly in order to provide the fish an environment which is free of pests and predators, and a pond bottom suitable for growth of natural food. Most of tilapia growers in Pampanga had an intensified stocking rate in order to replenish loss of stocks due to mortality. Increase in stocking rate beyond the biological capacity of the pond system could necessitate the total dependency in commercial feeds. Excess feed input and accumulated waste in the system could deteriorate both the water and soil. Roughly 25% of the phosphorus in aquafeeds is harvested in aquaculture biomass and the remainder is in uneaten feed and feces that microbially decompose and release phosphate, or metabolic excretions of culture animals [12].However, almost 80% of the unrecovered phosphorus in harvested fish is found in the pond bottom [13].

The municipality of Minalin was not only known for its tilapia production but also as egg granary in Luzon, Philippines. A number of poultry farms were located within the municipality. Some of the interviewed farms are near to existing poultry farms. A number of flies and birds in the poultry farms move in the tilapia ponds during and after harvest. Flies and birds can carry pathogenic organisms to the pond water through direct and indirect means.

Majority of the farms in Minalin, Pampanga, Philippines were not implementing any forms of biosecurity measures. Equipment was not disinfected after use and there was no designated area for their safe keeping. Free-ranged animals such as poultry, cats and dogs were found inside the farm. They can contaminate the feeds that were improperly kept in a storage room made of light materials. Dead fishes were just thrown elsewhere thus a possible mean of spreading bacterial diseases. Also, the roaming animals can feed on rotting tilapia carcasses.

**CONCLUSION**

The occurrence of bacterial diseases in tilapia is caused by the interplay of various risk factors such as presence of pathogenic organism and source of contamination, and a susceptible host due to stressful environment. Since wastewater from aquaculture and agriculture were dumped in the river system without any treatment, their main source of water could serve as reservoir of present and future problems on diseases. There is also a big possibility that a large percentage of the cultured tilapia from the unvisited farms during the time of investigation might be positive to *A. hydrophila*. The presence of disease vectors and carriers inside and outside the farm could magnify the present problem of tilapia operators in Minalin, Pampanga, Philippines.

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