Kor. J. Env. Hlth. Soc., Vol. 29, No. 4, pp 1~3(2003)

An Examination Performed to Identify the Causative Agent of Crusian Carp (*Carassius carassius*) Mass Mortality in Jinyangho

GeeWook Shin · HuJang Lee · TaeSung Jung†

Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 660-701, Korea

(Received August 28, 2003/Accepted October 15, 2003)

Abstract : Jinyangho is a natural water supply source of tap water in west Gyeongnam area, but mass mortality of crusian carp occurred during the time of temperature rise in spring. Examinations on diseased fishes were able to isolate four bacteria isolates and then identified the bacteria as a member of *Aeromonas* sp. Challenge experiment with mirror carp (*Gyrinus carpiospecilaris*) was proved the virulence, the isolates were in turn believed as the causative agent of mass mortality in Jinyangho.

Keywords : mass mortality, water supply source, crusian carp

Introduction

Jinyangho is an artificially constructed lake located in Jinju city, Gyeongnam. The lake collects all the water draining from Jiri mountain located at west Gyeongnam area which helps the lake maintain its clean and sufficient water as a natural source of water supply[1]. The water supply source is providing tap water to 17 Counties and Cities, such as Jinju City, Sacheon City, Tongyeon City, Namhae County, Hadong County, Geoje City, etc. The lake is usual not only for flood control reservoir and power generation, but also for supplying tap water to more than 200 million people in west Gyeongnam area. It is important that water supply source to use tap water is to maintain its good condition to be used as drinking water. Good condition means to satisfy both a variety of chemical and biological factors[2]. Between two factors, biological factors might be the easiest and best parameter to measure the safety of water. However, the importance of biological factors have been either ignored or considered less important in most routine examinations.

Recently, mass mortality of aquatic animal, especially cyprinids, occurred in Jingyangho during the time of temperature rise in spring, which made local peoples suspect the safety of the water. Therefore, the causative agent of mass mortality was investigated by collecting dead or moribund crusian carps (*Carassius carassius*), and challenge experiment was also performed with the isolates to evaluate the pathogens.

†Corresponding author : Institure of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University
Tel. 82-55-751-5822, Fax. 82-55-751-5803
E-mail : jungts@nongae.gsnu.ac.kr

Material and Methods

Samples

Based on the records of mass mortality occurred in Jinyangho at the end of May, the environmental Protection Bureau of Jinju city requested to investigate the cause of death on 10th of July, 2002. The extent of mortality was confirmed at the site and moribund fishes were collected on the next day. The external signs of fishes were highly suspected as bacterial infection. In turn, isolation of the infectious agents was performed from spleen, kidney and liver with tryptose soya agar (TSA). Another batch of sampling and trial were performed on 12th of July, 2002. Seven fishes were used to isolate the agent on the 10th of July, and three fishes were investigated on the 12th of July.

External and Internal signs

Diseased fishes were showed abdominal hem-

orrhages, necrosis on either abdominal wall or both sides, hemorrhages at pectoral and tail fins, but specific signs were not observed on head part. No other specific signs were observed on internal signs except on one fish showing hemorrhagic on abdominal cavity.

Isolation of bacteria

Three bacteria isolates were isolated among seven diseased fishes from sampled collected on 10th of July, and one isolate was isolated from three fishes collected on 12th of the same month.

Isolation and Identification of bacterial species

General bacteriological and biochemical examination

Gram staining, catalase and oxidase test (Oxoid inc.,), O-F test, Motility test with semisolid media, and metabolism test were performed according to the routine method of laboratory. API20E strip kit (Biomerieux) was used to test a total of 20 different carbohydrates being utilised, such as ONPG, ADH, H2S etc[3].

Challenge test

A total of 20 mirror carps (approximately 200 g) were divided into five groups. Respective volumes of 10^7 c.f.u/m*l*, 10^6 c.f.u/m*l*, 10^5 c.f.u/m*l*, and 10^4 c.f.u/m*l* of 02062 isolate grown with tryptose soy broth (TSB) were injected to 4 groups of fishes through I.P. One group of fishes was injected with PBS as a control. Bacterial isolation was performed from dead or moribund fishes, and examination was based on the above general bacteriological and biochemical methods.

Results and Discussion

Table 1 and 2 shows the results of general bacteriological and biochemical test. Dead fishes were collected from the group injected with 10^7 c.f.u/m*l* of 02062 isolate on 3, 5, and 7 days post injection, and groups injected with 10^5 and 10^6 c.f.u/m*l* were showed dead fishes on 4 and 5 days post injection. These dead fishes were showed abdominal hemorrhages as external signs, and presence of abdominal fluid and hemorrhages on organs were observed on some fishes as internal signs. The causative agents of mass mortality of aquaculture fishes were virus, bacteria, parasite and fungus. Cyprinids are also labile to infection by the agents. The agents of spring viraemia of carp (SVC) and carp epitheliomais are identified as *Rhabdovirus carpio* and *Herpesvirus of carp*[4].

Table 2. The result of biochemical test with API 20E Kit

API 20E	020601	020602	020603	020604
ONPG	+	+	+	+
ADH	+	+	+	+
LDC	+	+	+	+
ODC	–	–	–	–
CIT	+	+	+	+
H2S	–	–	–	–
URE	–	–	–	–
TDA	–	–	–	–
IND	+	+	+	+
VP	–	–	–	–
GEL	+	+	+	+
GLU	+	+	+	+
MAN	+	+	+	+
INO	–	–	–	–
SOR	–	–	–	–
RHA	–	–	–	–
SAC	+	+	+	+
MEL	+	+	+	+
AMY	–	–	–	–
ARA	–	–	–	–

Table 1. The result of general bacteriological test for isolates

Isolate	Gram staining	Morphology	motility	Catalase	Oxidase	OF test	O129 test
020601	–	rod	–	–	+	fermentation	–
020602	–	rod	–	–	+	fermentation	–
020603	–	rod	–	–	+	fermentation	–
020604	–	rod	–	–	+	fermentation	–

Carp erythrodermatitis (CE) are caused by *Aeromonas salmonicida nova* and *Aeromonas hydrophila,* while branchiomycosis was developed by fungus, *Branchiomyces sanguinis*[5]. Crytobia infection, coccidosis, and myxosporean infections were reported by occurring parasites[6]. Some of cyprinid diseases, especially viral disease, are reported to occur on spring regularly. At the end of winter, the hibernating carp has used most of its reserves and energy. Fish are highly weakened if there were strong cold winter or a mild season with relatively high temperature due to no feeding. As the water temperature rises in spring, energy requirements also increases but often natural food production has not yet started. Under these conditions, the carp is more susceptible since active immune reactions start only at higher winter temperature from about 14°C upwards. Therefore, it needs to consider other factors, such as the state of health, water temperature, and dissolved oxygen, in order to identify the causative agents of mortality caused by infection.

Four isolates from diseased fishes were showed similarities staining, morphology, and colony form, with the same biochemical reaction patterns. Among the fish pathogenic bacteria, *vibrio* sp., *plesiomonas* sp., and *aeromonas* sp. were showed gram negative, oxidase positive, and glucose fermentation. The isolates did not react with O129 test and degraded insitol. It was in turn identified as *aeromonas* sp[7]. The *aeromonas* sp. has been reported as a pathogen for a variety of freshwater fish, such as cyprinids, catfish, etc. Although challenge experiment with Mirror carp proved the virulence, isolates from Jingyangho was highly suspected as the causative agent of mass mortality. Different fish health status, water quality, and culture environments might play a role in lowering the virulence during challenge experiment. There are a couple of huddles in order that the isolated bacteria would believe as the causative agent of mass mortality. Firstly, more samples should be collected. Secondly, to determine whether or not the isolates in 2002 are the same bacteria identified in previous years. It is a very race case since Jinygangho is known to have a clean and good state of environmental and water source but still mass mortality occurred for years. Other facts, such as high density of fishes in the lake, weakened fish immune status, rapid increase of water temperature, and the existence of unidentified carrier, might be involved in increasing the virulence of the pathogen. It is necessary to give much attention to the water supply source to determine the primary cause of mass mortality in the future.

Reference

1. Korea water resouces coperation : Estimation of water quality after 2nd stage constuction of broad tap water with Namgan Dam (in Korean). Dajeon. 1996.
2. WHO : Guidelines of drinking water quality. 2nd Ed. Vol. 1 Recomnedation. 1996.
3. Valerie, I., Ronald J.R. and Niall, R.B. : Bacterial diseases of fish. Blackwell Science. 1993.
4. Wolf, K. : Grass carp reovirus. In Fish viruses and Fish viral Diseases, (ed. K. Wolf), pp. 65-68. Ithaca, NY: Cornell University Press. 1988.
5. József, F.S., Hiroshi, Y., Shigeyoshi, F. and Riichi, K. : Lymphomyeloid cells, susceptibility to erythrodermatitis of carp and bacterial antigens. *Dev. & Com. Immun.,* **14**, 185-200, 1990.
6. Erik, L.J. : Koi health and disease. Georgia. 1997.
7. Kimberley, A.W. and Neil, M. : Fish and shellfish "bacteriology manual". Fish Health Atlantic Veterinary College. 2001.