Characteristics of Infectious Dropsy from an Epizootic of Cultured Common Carp (*Cyprinus carpio L.*) With Special Investigation to Swim-Bladder Lesions

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Abstract:
Infectious dropsy was recorded in 2015 among a group of Common carp (*Cyprinus carpio* L.) collected from several private fish farms at Sharkiya Province, Egypt. Out of 200 clinical cases, 180 (90%) Common carp were found infected by bacteria. The bacterial isolates revealed a mixed infection with *Aeromonas hydrophila* and *Pseudomonas fluorescens*. The affected fish presented typical signs of infectious dropsy including hemorrhagic lesions in the skin, fin, tail, and eye. Loss of scale with sluggish movement and imbalance were also observed in some fish. Grossly, the diseased fish exhibited symptoms of septicemia such as congestion and hemorrhages in the skin and internal organs with distended anus, exophthalmia and ascites. The antimicrobial resistance of the isolated *bacteria* was higher with Oxytetracycline (OX) and lower with Ciprofloxacin (CIP). The other used antimicrobials showed variable resistance to both bacterial isolates. Experimental infection was done on 100 Common carp and revealed same clinical findings and gross lesions of the field study with 76 % mortality. Microscopically, the internal organs showed degenerative changes, focal necrosis, circulatory disturbances and inflammatory reactions. The swim bladder mucosa of infected carp exhibited necrosis, epithelial sloughing with congestion and lymphocytic infiltration. This study describes Infectious dropsy among cultured carp in Egypt and highlighted the importance of implementing preventive measures to control this infection.
Key words: *Common Carp, Infectious dropsy, bacteria, antibiotics, swim bladder.*

Introduction:
Aquaculture, in Egypt, express a fast development during the last decades with over 99 percent produced by private farms ***(FAO, 2012, GAFRD 2014).*** While this

growth is much appreciated in terms of food security, outbreaks due to disease hinder the development of aquaculture and have a negative impact on the economy not only in Egypt but also in many countries. Bacterial infections in fish are one of the challenges that influence sustainability of aquaculture production in Egypt and elsewhere ***(Aly 1994, Parvez and Mudarris, 2014).***

Infectious dropsy (ID) in common carp is one of the bacterial hemorrhagic septicemic disease that has been described by many investigators ***(Kumar and Dey (1991); Bohai et al., 1993).*** Definitive diagnosis of ID based on clinical findings and the detection of the etiology. Bacterial hemorrhagic septicemia is caused by *P. fluorescens* ***(Wakabayashi and Egusa, 1972 and Shiose et al., 1974)*** which act as a primary pathogen of freshwater fish and an opportunistic bacteria for variable marine and brackish waters fishes ***(Hadi et al., 2002; Alicia et al., 2004 and Foysal et al., 2011).*** Other studies mentioned that *A. hydrophila* may act as a primary pathogen for fish or secondary invaders for cases of hemorrhagic septicemia ***(Candan et al., 1995; Kozinska, 2002 and Güvener & Timur, 2005).*** Recently, *A. hydrophila* and *P. fluorescens* were recorded as the cause of Bacterial Hemorrhagic Septicemia (BHS) in *Cyprinus carpio* and *Channa striatus* ***(Parvez and Mudarris, 2014).*** The present work was, therefore, undertaken to identify the etiological agents of ID in Common carp (*Cyprinus carpio* L.) and to describe the symptoms and pathological lesions of the disease with special focus on the swim bladder.

Materials and methods

1. Fish and Sample collection:

In the Early spring of 2015 complex non-recognized disease occurred suddenly during the production stage of Common carp (*Cyprinus carpio* L.) in several fish farms at Sharkia Province. A total number of 200 clinically diseased common carp, weighed 50 – 150 g, of both sexes were collected as a random samples, transferred alive to the laboratory and kept in well-aerated glass aquaria at 25 °C and examined clinically using the methods described by ***Lucky (1977)*** with special attention to abnormal coloration, swimming behavior, respiratory manifestation, escape reflex and its appetite.

2. Bacteriological examination:

A total of 200 clinically diseased Common carp were collected and sacrificed by decapitation and disinfected with 70% ethanol. Bacteriological swab samples were taken from the cleaned surface of liver, spleen, kidneys and swim bladder after gentle removal of the superficial layer and sterilization of the exposed surface of the organ.

The bacteriological swabs were inoculated in Trypticase soya broth (TSB) and nutrient broth. The broth

cultures were aerobically incubated at 20-25 ºC for 18-24 hrs. A loopful of broth was cultured on selective media (Rimler-shotts agar, Ordal's Pseudomonas F. agar, Mac-Conkey's agar, trypticase soya agar). The inoculated plates were then incubated at 25-30 ºC for 24-48 hrs and colonies were picked up to nutrient slope agar and incubated at 25-30 ºC for 24-48 hrs. The isolated bacteria were identified via morphological as well as biochemical examinations as reported by ***Frerichs and Hendrie (1985) and Scheperclaus et al. (1992).***

3. Determination of antimicrobial resistance:

The resistance of *A. hydrophila* and *P. fluorescens*, that isolated in the present study, was tested through disk diffusion using The Mueller–Hinton agar (Difco.). Six antimicrobial agents were used [chloramphenicol C, (30 µg, < 13 mm); oxytetracycline, OX (30 µg, < 15 mm); ciprofloxacin, CIP (5 µg, < 16 mm); Kanamycin K, (30 µg, < 13 mm); Sulphamethoxazole/Trimethoprim, SXT (25 µg, < 11 mm), and Nalidixic acid, NA.(30 µg, < 14 mm)]. The sensitive isolates were differentiated from the resistant one through the use of break point values. All the used disks were purchased from Oxoid. The assays using disk diffusion were prepared based on the CLSI recommendations ***(CLSI, 2005 a & b).***

4. Experimental infections:

One hundred apparently healthy Common carp (150 ± 50 gm) were divided into 2 equal groups. Each group was reared in two glass aquaria each of 250 liters capacity. All Common carp were fed a balanced diet suitable for the given fish species and kept 2 weeks before the experiment for acclimatization and observation. The food ingredients were purchased from private suppliers and prepared in the form of pellets.

Fish of first group injected I/P with 0.5 ml (10^8 cells / ml) of equal mixture of isolated *A. hydrophila* and *P. fluorescens* from clinically infected Common carp. Second group act as a control injected I/P with 0.5 ml sterile broth. The injections were done according to ***Lucky, (1977) and Scheperclaus et al. (1992).*** All experimentally infected Common carp were noticed for clinical findings or mortalities throughout the experiment. The clinically diseased fishes were subjected to postmortem and histopathological examinations after two weeks post-infection. In addition, bacterial re-isolation was done.

5. Histopathological examination:

Specimens from the internal organs and swim bladder of experimentally infected and control Common carp were fixed in 10% neutral buffered formalin. Paraffin sections (5mu thick) were obtained and stained with hematoxylin and eosin (H&E) ***(Bancroft et al., 1996).***

Results

1. Field study:

A. Clinical findings:

The adult infected Common carp revealed exophthalmia and abdominal distension where the anal orifice was frequently protruded in addition to loss of response to the external stimuli in young infected Common carp. Hemorrhagic eyeballs together with sluggish movement and imbalance were also observed in some fishes.

B. Identification of the Isolates:

Out 200 clinically diseased Common carp, 180 fish (90%) were found infected by bacteria. The isolates were assessed for their morphological and biochemical characteristics. Among these, two isolates were pathogenic bacteria, the first isolate was Gram negative, rod shaped, motile with polar flagella, catalase positive, oxidative bacteria, able to ferment glucose identified as *A. hydrophila* (85.7%). The second isolate was Gram negative, rod shaped, motile with polar flagella, catalase positive, oxidative bacteria, produced acid from glucose in paraffin free media but unable to ferment glucose identified as *P. fluorescens* (84.3%).

C. Antimicrobial resistance:

The antimicrobial resistance of the isolated *A. hydrophila* and *P. fluorescens* was higher with *Oxytetracycline* (OX) and lower with *Ciprofloxacin* (CIP). The other used antimicrobials [*Chloramphenicol* (C), *Sulphamethoxazole/Trimethoprim* (SXT), *Nalidixic acid* (NA) & *Kanamycine* (K)] showed variable resistance to both bacterial isolates (Table, 1).

2. Experimental study:

The clinical signs, appeared in 96% of experimented common carp, were similar to the natural infection, it was observed within 10 – 24 hrs post-infection with *A. hydrophila* and *P. fluorescens*. The infected fish exhibited loss of appetite, loss of balance, upward position, intermittent hyperexcitation and finally loss of reflex prior to death. Mortality reached 76 % by the end of the observation period (2 weeks).

Grossly, the experimentally infected Common carp revealed loss of scales and petechial hemorrhages on the body surface and underlying muscles with distended abdomen (Fig. 1). The liver was enlarged with pale white necrotic foci on its surface, bloody fluid was seen in the pericardium and peritoneum together with congested kidneys and intestine (Fig. 2). The posterior chamber of the swim bladder found congested while the anterior chamber appeared whitish gray with petechial hemorrhages (Fig. 3).

Microscopically, the internal organs showed signs of septicemia where degenerative changes, focal necrosis, circulatory disturbances and inflammatory reactions in heart and liver were evident (Figs. 4 & 5). The swim bladder in some cases showed extensive necrosis in the epithelial lining with focal aggregation of melanomacrophage

cells in the subepithelial tissue. In other cases, the swim bladder showed mild congestion, edema and leukocytic infiltration in the submucosa and focal hyalinization in the muscle layer (Fig. 6). In the majority of cases, the swim bladder showed marked congestion with hemorrhage, diffuse necrosis and mononuclear cells infiltration (Fig. 7). The mucosa, submucosa and muscularis were focally replaced by marked fibrous connective tissue proliferation (Fig. 8).

The Common carp of the control group did not show any clinical signs of diseases with no gross or microscopic lesions.

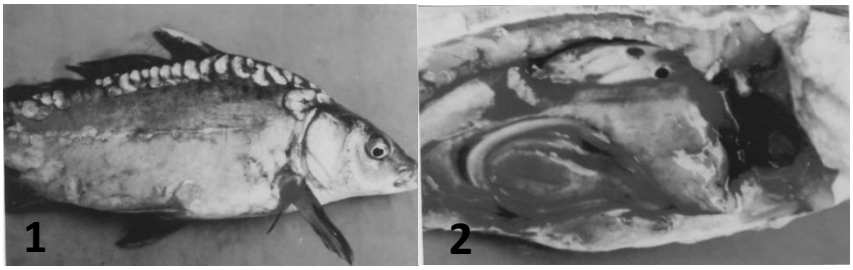

Fig. 1: Common carp post-infection with *A. hydrophila* and *P. fluorescens* showing loss of scales, petechial hemorrhages on the body surface and distended abdomen.

Fig. 2: Common carp post-infection with *A. hydrophila* and *P. fluorescens* showing enlarged pale liver white necrotic foci on its surface. Bloody fluid was seen in the body cavities together with congested kidneys and intestine.

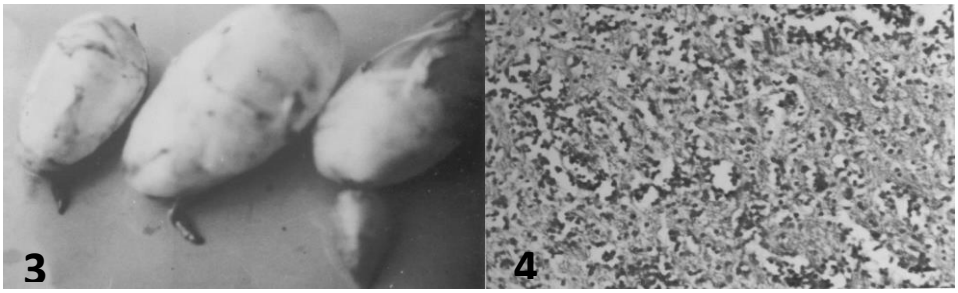

Fig. 3: Swim bladder of common carp post-infection with *A. hydrophila* and *P. fluorescens* showing congested posterior chamber and whitish gray hemorrhagic anterior chamber.

Fig. 4: Heart of Common carp post-infection with *A. hydrophila* and *P. fluorescens* showing focal necrosis and massive inflammatory reactions in the myocardium. H & E stain, x 250.

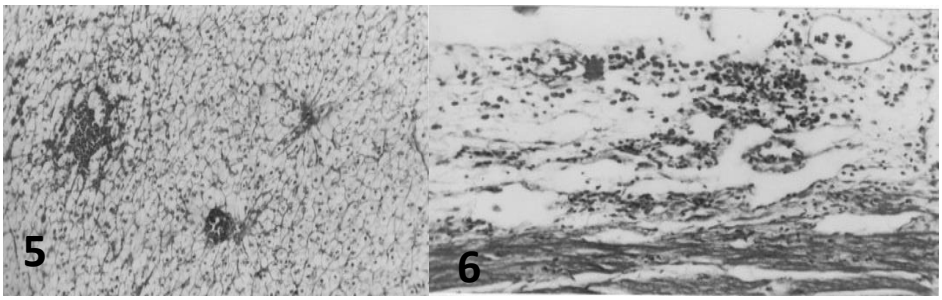

Fig. 5: Liver of Common carp post-infection with *A. hydrophila* and *P. fluorescens* showing vacuolar degeneration, focal necrosis and focal inflammatory reactions in the hepatic parenchyma. H & E stain, x 250.
Fig. 6: Swim bladder of Common carp post-infection with A. hydrophila and P. fluorescens showing extensive necrosis in the epithelial lining with congestion, edema and leukocytic infiltration in the submucosa. H & E stain, x 250.

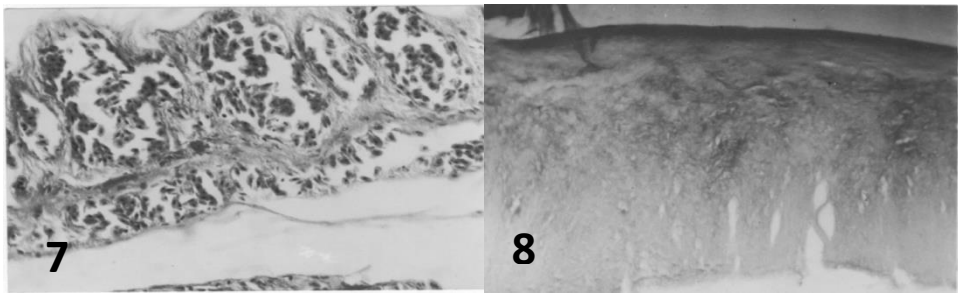

Fig. 7: Swim bladder of Common carp post-infection with *A. hydrophila* and *P. fluorescens* showing marked congestion and hemorrhage. H & E stain, x 250.
Fig. 8: Swim bladder of Common carp post-infection with *A. hydrophila* and *P. fluorescens* showing marked fibrous connective tissue proliferation in the submucosa and muscularis. Van Geisons stain, X 250.

Table (1): Resistance of *A. hydrophila* and *P. fluorescens* that isolated from the infected common carp to the tested antimicrobials.

Bacteria	Antimicrobial resistance (%)					
	C	SXT	CIP	NA	OX	K
A. hydrophila	61.00	57.67	00.00	46.67	73.33	53.22
P. fluorescens	66.00	75.33	21.67	85.33	100.00	97.00

C = Chloramphenicol, SXT = Sulphamethoxazole/Trimethoprim, CIP = Ciprofloxacin, NA = Nalidixic acid, OX = Oxytetracycline, K = Kanamycine.

Discussion

In the present study, the diseased fish was collected with expression of hemorrhagic lesions in the eye and skin together with sluggish movement, loss of balance and distended abdomen. These results attributed to the mixed bacterial infection and similar symptoms have also been reported by others ***(Kumar and Dey, 1991; Snieszko and Bullock, 1976; Moln´ar and Csaba, 2005 and Foysal et al., 2011).***

Bacteriological isolation from diseased carp revealed two pathogenic bacteriare exhibited the morphological and biochemical properties resembled of *A. hydrophila* and *P. fluorescens*. These isolates supported by several studies (***Miyashita, 1984; Joseph and Carnahan,1994; Candan et al., 1995; Kozinska, 2002; Güvener and Timur, 2005; Shiose et al., 1974; Hadi et al., 2002; Alicia et al., 2004; Foysal et al., 2011 and Parvez and Mudarris, 2014). Richards and Roberts (1978)*** pointed out that, *A. hydrophila* and *P. fluorescens* are ubiquitious in the aquatic environment and frequently implicated in the aetiology of bacterial hemorrhagic septicemia. ***Kozi´nska and Pe´kala (2012)*** reported that, all strains of *A. hydrophila* caused skin ulcers as well as septicaemia in carp where carp skin showed great susceptibility to infection of all Aeromonas strains. Recent study indicated that *A.hydrophila* and *P.fluorescens* were the etiological agents for bacterial hemorrhagic septicemic disease in *Cyprinus carpio* ***(Parvez and Mudarris, 2014).*** Additionally, it is reported that, Aeromonas spp. constitute very often the component of mixed bacterial flora isolated from asymptomatic carriers as well as from fish with various disease conditions caused sometimes by bacteria belonging to completely different taxa ***(Kozi´nska and Pe´kala, 2012).***

A high degree of resistance towards tetracyclines has been displayed by *P. fluorescens* (100%) and *A. hydrophila (73%)*. the percentage of tetracycline resistance in our study was consistent with several studies in aquatic culture ***(Penders and Stobberingh (2008); Schmidt et al., (2001); Petersen and Dalsgaard (2003) and Akinbowale et al., 2007).*** Although *A. hydrophila* and *P. fluorescens* displayed decreased susceptibility to the ^{1}st generation quinolones nalidixic acid they were highly susceptible to the newer generation ciprofloxacin which could be due to the recent use of ciprofloxacin in aquaculture. This result is consistent with that of ***Sreedharan et al., 2012***. In addition, our finding revealed the high resistance of both isolates to chloramphenicol which is consistent with that of ***Nguyen et al., (2014) and Chang et al., (2007).***

The isolation of bacteria from mixed bacterial flora does not

always indicate that they are primary factor of a disease, so experimental infection was carried out to reproduce the recorded field finding. The experimental study confirm that both bacterial isolates are able to produce the disease.

The gross pathological examination of the affected organs indicated necrotic foci in the liver, distended gall bladder and shrinkage kidney together with hemopericardium and hemoperitoneum. These pathological findings are in accordance with that reported by K***umar et al, (1986); Kumar and Dey (1986) and Rober et al., 2000.*** In relation to swim bladder, it appears that our paper is the first to describe the histopathology of the swim bladder in relation to challenge with mixed *A. hydrophila* and *P. fluorescens.*

Conclusion:

For understanding the disease process in mixed infection, external clinical symptoms together with histopathological changes are important. The findings presented in this study may be helpful in facilitating the diagnosis of bacterial hemorrhagic septicemia caused by *A. hydrophila* and *P. fluorescens* in *Common carp* but complementary studies are needed in order to better understand the pathogenesis of the disease and to set and implement preventive measures to control this disease in Egyptian aquaculture.

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خصائص الاستسقاء المعدي من وباء فى سمك المبروك العادي المستزرع مع فحص خاص للتغييرات المرضية فى المثانة الهوائية

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تم تسجيل مرض الاستسقاء المعدي عام 2015 بين مجموعة من المبروك العادي المجمعة من عدة مزارع للأسماك بمحافظة الشرقية. وبين 200 حالة اكلينيكية وجد 180 من المبروك العادي (90%) مصابة بالبكتيريا. وقد أبدت الاسماك المتأثرة بالأعراض الاكلينيكية للاستسقاء المعدي متمثله في أفات تزيفيه علي الجلد والزعانف والذيل والعين. كما شوهد فقدان لقشور الاسماك مع تعثر الحركة وفقدان الاتزان في بعض الاسماك. وبالفحص العيني اتصفت الاسماك المريضة بأعراض التسمم الدموي مثل الاحتقان والانزفة علي الجلد والأعضاء الداخلية مع بروز لفتحة الشرج وجحوظ للعينين واستسقاء. وقد تبين من العزل البكتيري وجود اصابة مشتركة بالايروموناس هيدروفيللا والسيدوموناس فلوريسنس وقد تبين ان البكتيريا المعزولة لديها مقاومة كبيرة للاوكسي تتراسكلين ومقاومة قليلة للسبروفلوكساسين وقد ظهر تباين في مقاومة البكتيريا المعزولة لمضادات الميكروبات المستخدمة. وقد اجريت عدوى تجريبية باستخدام 100 من المبروك العادى وتأكدت الاعراض الاكلينيكية والصفات التشريحية مع الدراسة الميدانية والتي نتج عنها 76% نفوق. وقد تبين بالفحص الميكروسكوبي وجود تغييرات هدامة وتخر موضعي واضطرابات دورية وتفاعلات التهابية بالأعضاء الداخلية. وقد ظهر علي غشاء المثانة الهوائية للمبروك المصاب نخر وفقدان للغشاء المبطن مع احتقان وارتشاحات بالخلايا الليمفاوية.

وقد وصفت هذه الدراسة الاستسقاء المعدي بين المبروك المستزرع في مصر وتوجيه الضوء لأهمية تطبيق الاجراءات المانعة للسيطرة علي هذه الاصابة.