# Studies on yersiniosis in cultured *Mugil seheli* for the first time in Suez Governorate

**Eissa, I.A.M[*]; El-Ashram, A.M.M[**] and Yasmine, A. El-Barbary[**]**

[*] Fish Diseases and Management Dept. Faculty of Vet. Med., Suez Canal University

[**] Fish Health and Diseases Dept. Faculty of Fish Resources, Suez University, Egypt.

## Abstract

A total of 360 cultured *Mugil seheli* had clinical signs were collected from private farms in Suez Governorate, Egypt. *M. seheli* was subjected to clinical, post -mortem and bacteriological examinations for detection of *Yersinia ruckeri*. *Yersinia ruckeri* was identified by biochemical reactions and polymerase chain reaction (PCR). One isolate of *Y. ruckeri*. The results revealed that the presence of *Yersinia ruckeri* infection was 68.1%. The highest prevalence of *Y. ruckeri* infection in cultured *M. seheli* was during autumn season (80%) followed by spring (72.2%), summer (70%) and then winter (50%). PCR amplification of DNA from *Y. ruckeri* isolates using 16s rRNA (YER8/10) specific primers for *Y. ruckeri* resulted in PCR products size of 575 bp. All *Y. ruckeri* isolates were found to be sensitive to Ciprofloxacin, Tobramycin and Trimethoprim. While resisted Erythromycin, Amoxycillin and Novobiocine.

## Introduction

Fish constitute a major source of protein, fatty acids, vitamins, minerals and essential micronutrients for an expanding segment of the world population. Consequently, aquaculture is the fastest growing food production sector and accounts for approximately 50% of the fish consumed worldwide (**Mathiesen, 2015**). *Mugil seheli* (*M. seheli*) is recognized as economically-important marine and brackish water fish and abundantly cultured in Suez Governorate, Egypt, for domestic consumption. *M. seheli* may be an excellent candidate for aquaculture especially in North Egypt regions because of its nutritional value.

Disease outbreaks have become a major constraint to the expansion of aquaculture and have a significant impact on the economic development of many countries. Bacterial pathogens are among the most important serious fish diseases in aquaculture causing 80% of fish mortalities **(Woo and Bruno, 1999).** Most of the bacteria, associated with these diseases, are saprophytic and widely distributed in the aquatic environment **(El-Ashram and Abd El-Rahman 2006; Austin and Austin 2007 and**

**Plumb and Hanson 2011).** Enteric redmouth disease (ERM, yersiniosis) is one of the most important diseases of salmonids and leads to significant economic losses (**Horne and Barnes 1999**). The disease is caused by *Yersinia ruckeri*, It's a Gram-negative rod-shaped enterobacterium, which was first isolated from rainbow trout (*Oncorhynchus mykiss*) in USA (**Ross *et al.*, 1966)** and is currently found throughout North and South America, Europe, Australia, South Africa, the Middle East and China (**Tobback *et al.*, 2007** and **Shaowu *et al.*, 2013)**.

16s rRNA gene (YER8/10) primer was used to identify *Y. ruckeri* and gave a 575 bp band when tested by means of PCR using these primers **(Eissa *et al.*, 2008)**.

The aim of the current investigation was to throw light on detection of *Y. ruckeri* in cultured *M. seheli* in Suez Governorate, Egypt with special reference to detection of 16s rRNA gene as diagnostic tool.

## MATERIALS AND METHODS

Naturally infected 360 *M. seheli* were collected from private fish farms in Suez Governorate using gill nets method described by **Portt (2006).** The average body weight and length were 39.11gm and 15.17cm respectively. Fish samples were subjected to clinical, post-mortem and bacteriological examinations **(Noga, 2010**, **Meyers, 2006** and **Austin and Austin, 2007)**. The prevalence of *Y. ruckeri* seasonally infected fish and organs also were recorded.

### Clinical and Postmortem Examinations:

Clinical and post-mortem examinations were carried out according to **Noga, (2010)** and **Meyers, (2006**).

### Isolation and identification of *Y. ruckeri*

Under complete aseptic condition, samples of gills and internal organs (liver, kidney and spleen) were collected from diseased *M. seheli* and cultivated on tryptic soy agar **(Micromaster)** ® supplemented with 1% NaCl and incubated at 25℃ for 24-48 hours **(Buller, 2014).** Colonies from general media were streaked on Yersinia selective agar base **(Micromaster)** ® supplemented with *Yersinia* selective supplement. The plates were incubated at 25℃ for 24 hrs. Separated colonies were described and pure culture was identified using phenotypical and biochemical characters according to The biochemical tests were used for identification of bacterial isolates.

### DNA extraction and PCR assay

Genomic DNA was extracted from bacterial isolates as described by **Devi *et al.*, (2009).** The broth culture of bacterial isolates incubated at 37 °C for 16–18 hrs, Then centrifuged (10,000 rpm, 1min) to obtain a pellet, which was then washed with normal saline (0.85w/v) and suspend pellet of cells in sterile distilled water (0.5 ml). Suspended pellets were placed in boiling water bath at 98±2 °C for 15–20 min in a water bath to lyse the cells. The lysate was centrifuged to remove the cell debris (10,000 rpm, 5 min). The supernatant was obtained and stored (−20 °C) until further use. Primer specific for 16s rRNA of *Yersinia ruckeri* was used for PCR protocol under specific condition which was done twice. The first one was done exactly according to **Gibello *et al.*, (1999)** and the second trial was achieved as previous but with some modification of the annealing temperature. The modification was 25 cycles of denaturation for 1 min at 92°C,annealing was raised to 62ċ for 1 min, and extension for 1 min at 72°C, followed by a final extension step of 72°C for 5 min. PCR amplified product was subjected to Gel electrophoresis in 1.5% (w/v) agarose gel**,** using TAE (tris-acetate-EDTA) as running buffer and the power was supply adjusted at 100 volt for 20-30 minutes **(lee *et al.*, 2012)**.

**Challenge test**

A total of 20 apparently healthy acclimated *M. seheli* were divided into two equal groups in glass aquaria filled with pond water supplemented with continuous aeration using electrical pump for a week before injection. First group injected intraperitoneally with 0.1 ml of *Y. ruckeri* $5 \times 10^5$ CFU **(Berc *et al.*, 1999)**. The second group injected with sterile saline by using one ml insulin syringe. The experimentally infected fish were daily observed up to 14 days for any abnormal clinical signs and mortalities. Mortality rate was calculated for each group and re-isolation of injected bacteria was done.

**Sensitivity test to antibiotic**

A total of randomly selected five *Y. ruckeri* isolates recovered from naturally infected *M. seheli* were used in antimicrobial susceptibility test. Susceptibility was determined by the disc-diffusion technique on Mueller-Hinton agar plates **(CLSI, 2013).** *Y. ruckeri* pure cultures were grown in brain heart infusion (BHI) broth for 24 hours at 37°C (**Samal *et al.*, 2014**). The suspension was swabbed on Mueller-Hinton medium plates. Discs were placed on inoculated plates and incubated for 24 hours at 37°C (**Thakur *et al.*, 2003** and **Aravena-Román *et al.*, 2012**). The antibiotic discs were used Amoxicillin, Ciprofloxacin, Erythromycin, Naldixic Acid, Novobiocin, Tobramycin and Trimethoprim. Isolates were

judged as sensitive (S), intermediately resistant (I) or resistant (R) on the basis of the size of the zone of bacteria growth inhibition according to the guidelines of the **CLSI (2013).**

## Results

### Clinical findings

The most common findings were hemorrhages on skin at different parts of the body, hemorrhages at the base of fins, on operculum and anal opening, swelling of abdomen **(Plate 1, A).** Some fish exhibited sluggishness, darkening of the fish coloration, swimming near the water surface with air gasping and bulging eyes. Affected fish showed loss of equilibrium and exhibited abnormal swimming behavior.

### Post- mortem findings

The post-mortem finding of naturally infected *M. seheli* showed petechial hemorrhages of the most internal organs and erythematous intestinal mucosa **(Plate 1, B)**. In other cases, the intestines voided of feed and contained yellowish mucus. Congested and enlarged spleen also was observed **(Plate 1, C)**. Moreover, enlarged and congested kidney and liver were also noticed.

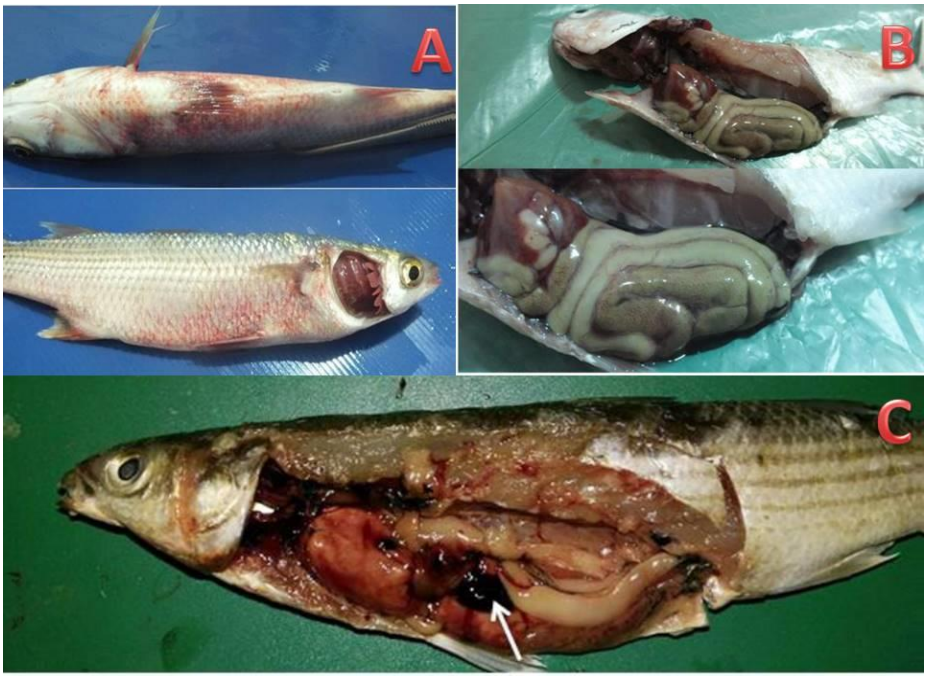


**Plate (1):** Naturally infected *M. seheli* showing hemorrhages on external body surface **(A),** petechial hemorrhages on intestinal external surface **(B)** and enlarged and congested spleen (arrow) and pale liver with hemorrhages **(C).**

**Bacteriological examination**

The phenotypic and biochemical characters of isolated bacteria from naturally infected *M. seheli* were illustrated in (**Table** 1).

**Morphological and biochemical identification (Table 1)**

**Table (1) Morphological and biochemical identification of *Y. ruckeri***

| Test | Result |
|---|---|
| **Gram staining** | - |
| **Colonies character on Yersinia selective agar base** | Pink colonies with red center |
| **Motility** | + |
| **Oxidase** | - |
| **Catalase** | + |
| **Ornithine decarboxylase** | + |
| **Indol** | - |
| **$H_2S$** | - |
| **Glucose fermentation** | + |
| **Citrate utilization** | + |
| **Gelatin liquification** | - |
| **Methyle red** | + |

**Prevalence of *Y. ruckeri* isolates in naturally infected *Mugil seheli*:**

**Total prevalence of *Y. ruckeri* infection in naturally infected *M. seheli*:**

The total prevalence of *Yersinia ruckeri* infection was 68.1% among the examined fish.

**Seasonal prevalence of *Y. ruckeri* infection in naturally infected *M. seheli*:**

The prevalences of *Y. ruckeri* infection in cultured *Mugil seheli* were 80% in autumn followed by 72.2% in spring, 70% in summer and 50% in winter.

**Prevalence of *Y. ruckeri* infection in organs of naturally infected *M. seheli*:**

The majority of *Yersinia ruckeri* isolates were recovered from gills 41.7% followed by liver 29.55% and kidney 25.1%.While the lowest percentage was recovered from spleen 3.64%.

**Experimental infection of *M. seheli*** with *Y. ruckeri*

The mortality rate of experimentally infected M. seheli with *Y. ruckeri* isolate was showed in **(Table 2)**. The most cases of experimentally infected fish suffered from abnormal swimming, darkening of the skin and loss of equilibrium within 24hrs post-injection. Also, severe diffused external hemorrhages on the body of experimental infected fish, redness of all fins in some cases; severe congestion and redness of the mouth were observed **(Photo 1)**.

**Photo (1) *Mugil seheli* artificially infected with *Yersinia ruckeri* showed sever hemorrhage of the mouth area.**

**Table (2): Results of pathogenicity of *Y. ruckeri* used in challenge test**

| Injected bacteria | No. of infected fish/control | Average weight of fish | Dose (CFU/Fish) | Method of infection | No. of dead fish | Mortality (%) | Post-infection days of mortality |
|---|---|---|---|---|---|---|---|
| *Y. ruckeri* | 10 | 30.5g | $5 \times 10^5$ | Intraperitoneal | 6 | 60 | 3-7 |
| Control | 10 | | 0.1 ml saline | | 0 | 0 | - |

**Molecular identification by PCR**

Tested seven bacterial isolates gave the expected bands of 575 bp size in lane one to seven when the *Y. ruckeri* specific 16s rRNA primers were used for molecular identification **(Photo 2)** by modified protocol **(Table 3,4)**.

**Table (3) Specific primers of *Y. ruckeri*, Annealing temperature and number of cycles used in DNA amplification of each isolate:**

| Bacteria | Name of primers | Gene product | Forward/ Reverse | AT | C |
|---|---|---|---|---|---|
| ***Yersinia ruckeri*** | **YER 8, YER 10** | **16s rRNA** | **F:GCGAGGAGGAAGGGTTAAGTG R: GAAGGCACCAAGGCATCTCTG Gibello *et al.*, 1999** and **Altinok 2001** | **62̊c** | **25** |

AT= Annealing temperature C= Number of cycles

**Table (4) Standard MyTaq ™ Mix Protocol**

| | |
|---|---|
| **Template** | **200ng** |
| **Forward primers (20pmol)** | **1µl** |
| **Reverse primers (20pmol)** | **1µl** |
| **MyTaq Mix, 2x** | **25µl** |
| **Water** | **Up to 50µl** |

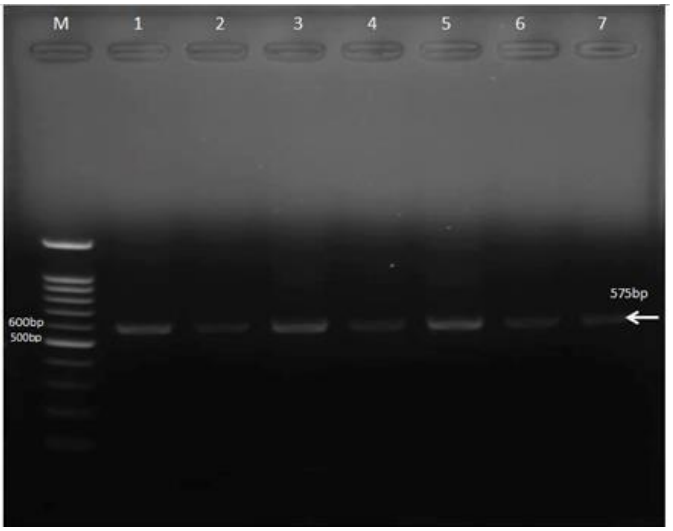


**Photo (2)** PCR amplification of DNA from *Yersinia ruckeri* isolates using specific primers for *Y. ruckeri* resulted in PCR products size of 575 bp.

**Antibiogram sensitivity of *Y. ruckeri***

All *Yersinia ruckeri* isolates were sensitive to Ciprofloxacin, Tobramycin and Trimethoprim. While resisted Erythromycin, Amoxycillin and Novobiocine (**Table 5**).

**Table (5) Antibiogram profile of *Y. ruckeri***

| Antibiotic | Break point (mm) | | | Average inhibitory zone of isolates (mm) | Sensitivity of isolates |
|---|---|---|---|---|---|
| | S | I | R | | |
| **Aml (10µg)** | >18 | 14-17 | <13 | 10 | R |
| **Cipro (5µg)** | >31 | 21-30 | <20 | 45 | S |
| **E (15µg)** | >23 | 14-22 | <13 | 10 | R |
| **NA (30µg)** | >19 | 14-18 | <13 | 10 | R |
| **NV (30µg)** | >22 | 18-21 | <17 | 10 | R |
| **TMP (5µg)** | >16 | 11-15 | <10 | 35 | S |
| **TOB (10µg)** | >15 | 13-14 | <12 | 16 | S |

Aml = Amoxycillin, cipro =ciprofloxacin, E= erythromycin, NA= naldixic acid, NV= novobiocin, TMP= trimethoprim, TOB= tobramycin, S= sensitive, I= intermediate resistant, R= resist.

## Discussion

The most common clinical signs of naturally infected fish were swimming near the surface of water, congested or pale gills, hemorrhages at the base of fins, abdominal part and gill cover, hemorrhages around the vent and mouth and some fish displayed abdominal distension. Similar picture was noticed by **Oren (1981)**, **El-Ashram** and **Abd El-Rahman (2006)** and **Enany *et al.*, (2011)**.

The results of postmortem examination of naturally infected fish revealed pale liver in some cases and in other cases liver were hemorrhagic. Kidneys and spleen were enlarged and congested. These results went hand in hand with that observed by **El-Ashram and Abd El-Rahman (2006) and Enany *et al.*, (2011)**.

Regarding to the bacteriological examination, *Yersinia ruckeri* was Gram negative short rod bacilli, grew on *Yersinia* selective agar base that not agreed with **Buller, (2014)** who reported that *Yersinia ruckeri* didn't grow on *Yersinia* selective agar base. *Yersinia ruckeri* in present study was oxidase negative and catalase positive. It was negative for hydrogen

sulphide production, indol production and gelatin liquefaction tests while positive for citrate utilization, ornithine decarboxylase and methyle red test. This finding was supported by **Altun *et al.*, (2013)**.

The mortality rate of *Yersinia ruckeri* in experimentally infected *M. seheli* fish was 60% in the present study. This result was nearly in line with that obtained by **Ohtani *et al.*, (2016)** who reported that the mortality of *Y. ruckeri* IP experimentally rainbow trout (*Oncorhynchus mykiss*) was 64%.

*Yersinia ruckeri* PCR assay using YER 8, YER 10 primers according to **Gibello *et al.*, (1999)** showed that the use of annealing temperature 60° C as indicated by them led to the appearance of non-specific bands. Therefore the temperature of annealing step was raised to 62° C in the present study (higher than that used by **Gibello *et al.*, 1999)** to avoid the appearance of non-specific bands, bacterial isolates gave the expected bands of 575 bp.

*Yersinia ruckeri* was isolated from cultured *Mugil seheli* which reared in brakish water in Suez governorate, **Toranz, (2004)** cited that *Yersinia ruckeri* was documented in cultured fresh and seawater fish. The total prevalence of *Yersinia ruckeri* in cultured *Mugil seheli* was 68.1%. These results were nearly in line with that obtained by **Eissa *et al.*, (2008)** who reported the prevalence of *Y. ruckeri* infection in cultured Nile tilapia, *Oreochromis niloticus* in Egypt was 66.6%.While **Aly (2013)** mentioned that incidence of *Y. ruckeri* in grey mullet was 20%.

All *Y. ruckeri* isolates were found to be sensitive to Ciprofloxacin, Tobramycin and Trimethoprim. While resisted Erythromycin, Amoxycillin and Novobiocine, these resulted supported by **Akhlaghi and Sharifi Yazdi (2008)** and **Lazar *et al.*, (2010)**.

It could be concluded that yersiniosis is one of the most important bacterial disease of cultured *M. seheli* and leads to significant economic losses. Ciprofloxacin, Tobramycin and Trimethoprim are the drugs of choice to control the disease. PCR is most sensitive and rapid method for identification *Yersinia ruckeri* pathogen.

## REFERENCES

**Akhlaghi, M. and Sharifi Yazdi, H., (2008):** Detection and identification of virulent Yersinia ruckeri: the causative agent of enteric redmouth disease in rainbow trout (Oncorhynchus mykiss) cultured in Fars province, Iran. Iranian Journal of Veterinary Research, 9(4), pp.347-352.

**Aravena-Román, M., Inglis, T. J., Henderson, B., Riley, T. V., and Chang, B. J. (2012):** Antimicrobial susceptibilities of *Aeromonas* strains isolated from clinical and environmental sources to 26 antimicrobial agents.Antimicrobial agents and chemotherapy, 56(2), 1110-1112.

**Altun, S., Onuk, E. E., Ciftci, A., Duman, M., & Büyükekiz, A. G. (2013):** Determination of Phenotypic, Serotypic and Genetic Diversity and Antibiotyping of Yersinia ruckeri Isolated from Rainbow Trout. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 19(2), 225-232.

**Aly, S. M. (2013**): A review of fish diseases in the Egyptian aquaculture sector: Working report. CGIAR Research Program on Livestock and Fish, 1-25

**Aravena-Román, M., Inglis, T. J., Henderson, B., Riley, T. V., and Chang, B. J. (2012):** Antimicrobial susceptibilities of *Aeromonas* strains isolated from clinical and environmental sources to 26 antimicrobial agents.Antimicrobial agents and chemotherapy, 56(2), 1110-1112.

**Austin, B., and Austin, D. A**. **(2007):** Bacterial fish pathogens: Disease of farmed and wild fish. Fourth edition. Springer Science & Business Media.

**Berc, A., Petrinec, Z., Matašin, Ž. & Kozarić, Z**. **(1999):** *Yersinia ruckeri* septicaemia in experimentally infected carp (Cyprinus carpio L.) fingerlings. Acta Veterinaria Hungarica, 47(2), 161-172.

**Buller, N. B**. **(2014):** Bacteria and Fungi from Fish and other Aquatic Animals: a practical identification manual. CABI. page 15,16,114,128,169,235,446 and706.

**Clinical and Laboratory Standards Institute (CLSI)** (2013): Informational Supplement; CLSI document M100-S21,Wayne, PA, USA. Vol. 31 No. 1 Pages13, 43 to 46.

**Devi, R., Surendran, P. K., and Chakraborty, K**. **(2009):** Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from shrimp farms along the southwest coast of India. World Journal of Microbiology and Biotechnology, 25(11), 2005-2012.

**Eissa, A. E., Moustafa, M., Abdelaziz, M., and Ezzeldeen, N. A. (2008):** *Yersinia ruckeri* infection in cultured Nile tilapia, *Oreochromis niloticus*, at a semi-intensive fish farm in Lower Egypt. African Journal of Aquatic Science, *33*(3), 283-286

**El-Ashram, A. M. M., and Abd El-Rahman, A. M. M. (2006):** Studies on bacterial diseases infecting mullet (*Mugil capito*) cultured in freshwater farms in Sharkia province. J. Agric. Res., 84 (1B) 461-471.

**Enany, M . EL., Ibrahim, H. M., Abou El Atta, M. El. and El Adawy, M. (2011):** Bacteriological and histopathological studies on some bacterial pathogens causing diseases in cultured *Mugil capito* fish in Ismailia governorate. Suez Canal Veterinary Medicine Journal, XVI. 1-12

**Gibello, A., Blanco, M. M., Moreno, M. A., Cutuli, M. T., Domenech, A., Dominguez, L., and Fernández-Garayzábal, J. F. (1999):** Development of a PCR Assay for Detection of *Yersinia ruckeri* in Tissues of Inoculated and Naturally Infected Trout. Applied and Environmental Microbiology, 65(1), 346-350.

**Horne, M.T; Barnes, A.C (1999):** Enteric redmouth disease (*Yersinia ruckeri*). Woo PTK, Bruno, Fish diseases and disorders. Viral, bacterial and fungal infections. CABI Publishing, Wallingford, pp 445–477

**Lazar, M., Vulpe, V., Guguianu, E., Lazar, R., Sarli, G., and Oprean, O. Z. (2010):** Red Mouth Disease in Rainbow Trout (Oncorhynchus mykiss)-a Case Report on Lake Trout Farm from Bicaz, Romania. Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Veterinary Medicine, 67(1).

**Lee, P. Y., Costumbrado, J., Hsu, C. Y., and Kim, Y. H. (2012):** Agarose gel electrophoresis for the separation of DNA fragments. JoVE (Journal of Visualized Experiments), (62), 3923. Advance online publication. http://doi.org/10.3791/3923.

**Mathiesen, Á. M. (2015**): The state of world fisheries and aquaculture 2012. Food and Agriculture Organization of The United Nations. 978-92-5-107225-7

**Meyers, T. R. (2004):** Standard necropsy procedures for finfish, second edition. National Wild Fish Health Survey–Laboratory Procedures Manual

**Noga, E. J. (2010):** Fish disease Diagnosis and Treatment. Mosby-yearbook, Inc. watsworth publishing Co., USA. pp.366.

**Ohtani, M., Strom, H. K., Lauritsen, A.H., Villumsen, K.R., Dalsgaard, I., Raida, M.K and Bojesen, A.M. (2016):** Influence of the challenge route on the pathogenicity of *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*). International Conference Organized by the European Aquaculture Society in cooperation with Marine

Scotland and The Marine Alliance for Science and Technology for Scotland, September 20-23, 2016, Edinburgh, Scotland.

**Oren, O. H. (1981**): Aquaculture of grey mullets. (Vol. 26). Cambridge University Press, U.K. pp. 1-19.

**Plumb, J. A., and Hanson, L. A. (2011):** Health maintenance and principal microbial diseases of cultured fishes. John Wiley & Sons.

**Portt, C. B., Coker, G. A., Ming, D. L., and Randall, R. G. (2006):** A review of fish sampling methods commonly used in Canadian freshwater habitats. Can. Tech. Rep. Fish. Aquat. Sci. 2604 p

**Ross, A.J; Rucker, R.R and Ewing, W.H (1966):** Description of a bacterium associated with redmouth disease of rainbow trout (*Salmo gairdneri*). Can J Microbiol 12:763–770

**Samal, S. K., Das, B. K., and Pal, B. B. (2014):** Isolation, biochemical characterization, antibiotic susceptibility study of *Aeromonas hydrophila* isolated from freshwater fish. Int. J. Curr. Microbiol. App. Sci, 3(12), 259-267.

**Shaowu, L; Di, W; Hongbai, L and Tongyan, L (2013):** Isolation of *Yersinia ruckeri* strain H01 from farm-raised amur sturgeon *Acipenser schrencki* in China. J Aquat Anim Health 25:9–14

**Thakur, A. B., Vaidya, R. B., and Suryawanshi, S. A. (2003):** Pathogenicity and antibiotic susceptibility of *Vibrio* species isolated from moribund shrimps. Indian journal of marine sciences, 32(1), 71-75

**Tobback, E; Decostere, A; Hermans, K; Haesebrouck, F and Chiers, K (2007):** *Yersinia ruckeri* infections in salmonid fish. J Fish Dis 30:257–268

**Toranzo, A. E. (2004):** Report about fish bacterial diseases. Mediterranean Aquaculture Laboratories, ed. Alvarez-Pellitero P, Barja JL, Basurco B, Berthe F, Toranzo AE, 49-89.

**Woo, P. T. K. and Bruno, D. W. (1999):** Fish diseases and disorders.Vol. 3,Viral, Bacterial and Fungal infections. CABI Publishing, London, U.K.

# دراسات عن مرض الفم الأحمر المعوى لأول مرة فى أسماك السهلية المستزرعة بمحافظة السويس

**اسماعيل عبد المنعم عيسي* أحمد محمد الأشرم** ياسمين أحمد البربري****

*** قسم أمراض الأسماك ورعايتها – كلية الطب لبيطري – جامعة قناة السويس.**

## الملخص العربى

أُجريت هذه الدراسة على (٣٦٠) سمكة من أسماك السهلية متوسط أوزانها (٣٩,١١ جرام) و متوسط أطوالها (١٥,١٧ سم) و المجمعة من المزارع الخاصة بمحافظة السويس بمصر فى الفترة ما بين نوفمبر ٢٠١٤ إلى أكتوبر ٢٠١٥، خضعت العينات للفحص من حيث الفحص الإكلينيكي و الصفة التشريحية المصاحبة للإصابة و الفحوص البكتيرية لعزل بكتيريا (يرسينا روكري) وإستخدام تقنية البلمرة المتسلسل في التشخيص.

أظهرت العلامات المرضية للأسماك المصابة وجود أنزفة مختلفة الأحجام ومنتشرة علي السطح الخارجي للسمكة ووجود أنزفة حول الفم و الفتحة الشرجية و إنتفاخ البطن و تساقط القشور مع ظهور قرح. بعض الإسماك أظهرت فقدان الشهية، دكانة لون الجسم، العوم بكسل، السباحة بالقرب من سطح الماء، تآكل الزعانف و جحوظ العين، فقدان التوازن و العوم بصورة عصبية.

تمثلت الصفة التشريحية للإسماك المصابة فى وجود سائل استسقائي، أنزفة على معظم الأحشاء و التهاب الأمعاء مع خلوها من الغذاء و تحتوى على مخاط أصفر اللون و إحتقان الخياشيم و المناسل و الطحال، بعض الأسماك أظهرت شحوب الخياشيم و الكبد مع ظهور أنزفة وضخامة و إحتقان في الكلي و الكبد.

تم تصنيف البكتريا من خلال إستخدام صبغة الجرام و زرع البكتيريا على البيئة البكتيرية الخاصة باليرسينيا، وكذلك تم دراسة الخواص البيوكيميائية للعزلات. و قد سجلت الدراسة أن معدل إصابة اسماك السهلية ببكتيريا اليرسينيا روكري٦٨,١٪ ، و تبين ان أعلي نسبة من اليرسينيا روكرى تم عزلها من الخياشيم ٤١,٧٪ يتبعها الكبد ٢٩,٥٥٪ ثم الكلي ٢٥,١٪ و اخيرا الطحال ٣,٦٤٪. و تم تأكيد نتائج العزل و التصنيف للبكتيريا بإستخدام إختبارالبلمرة المتسلسل لعزلات اليرسينيا روكري بإستخدام برايمر خاص لليرسينيا روكرى.

أوضحت نتائج العدوى المعملية لإسماك السهلية المستزرعة باليرسينيا روكري انها تسبب النفوق فى الأسماك خلال ٧ يوم، و تلخصت العلامات المرضية فى ظهور حركات دائرية، دكانة لون الجلد و فقدان التوازن و ذلك بعد ٢٤ ساعة من إجراء العدوى، أيضاً ظهور تآكل الزعانف و أنزفة علي قاعدة الزعانف و أجزاء مختلفة من الجسم، إحتقان الفتحة الشرجية مع أنزفة، تساقط شديد فى القشور مع بداية ظهور قرح و إحتقان و إحمرار فى منطقة الفم. قد بينت الدراسات أن الصفات التشريحية لأسماك العدوى المعملية تلخصت فى إحتقان فى الكبد مع وجود أجزاء شاحبة ، إحتقان و زيادة في حجم الكلي، أنزفة على جدار الأمعاء و زيادة في حجم الحوصلة المرارية.

تم إجراء إختبار الحساسية لعزلات اليرسينيا روكرى للمضادات الحيوية وأوضحت النتائج أنها حساسة للسيبروفلوكساسين و الترايميثوبريم و التوبراميسين و مقاومة للنوفوبيوسين ،أيرثروميسين، حامض النالدكسيك وأموكسيسلين.