YERSINIOSIS OF FRESHWATER FISHES IN SOHAG, UPPER EGYPT

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ABSTRACT

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The current study was carried out to investigate *Y. ruckeri* infection among *O. niloticus* (Nile tilapia) and *C. gariepinus* (African catfish) at Sohag Governorate. Ninety-three samples of *O. niloticus* and eighty-seven samples of *C. gariepinus* were collected from different localities at Sohag Governorate during the period from March 2014 to March 2015. Fish samples were subjected to clinical, post-mortem examination and bacteriological examination. The liver, kidney and spleen were chosen for bacteriological examination. The suspected isolates were characterized by cultural and morphological characters, some conventional biochemical tests and API 20E system. Five isolates were characterized as *Y. ruckeri* [3 isolates (Y1-Y3) from *O. niloticus* (3.2%) and 2 isolates (Y4-Y5) from *C. gariepinus* (2.3%)]. The phenotypic characterization of these isolates revealed that they were homogenous except in Voges-Proskauer test and gelatine liquifaction, the similarity between them was ranged from 92.9 to 100% and they belonged to Biotype 1 of *Y. ruckeri*.

Keywords: *Yersinia ruckeri, Oreochromis niloticus, Clarias gariepinus, isolation and identification.*

INTRODUCTION

In Egypt, *O. niloticus* and *C. gariepinus* are the two main species reared in aquaculture (Ibrahem *et al*., 2011). *Y. ruckeri* causes enteric redmouth disease which is a serious bacterial septicaemia in salmonids and other fish species of commercial importance worldwide (Furones *et al*., 1993). *Y. ruckeri* presents in a carrier state in many fish species and remains undetected until stressors particularly associated with intensive culture and poor water quality where may result in heavy losses requiring immediate intervention (Horne and Barnes, 1999). *Y. ruckeri* strains can be divided into two biotypes, biotype 1 and 2 based on motility, Lipase activity, VP reaction, and the ability to hydrolyse Tween (Tinsley *et al*., 2010) and they are serologically diverse (Austin and Austin, 2007). There is little phenotypic variation between *Y. ruckeri* strains other than that present in the two biotypes within serogroup O1 (Davies and Frerichs, 1989).

Y. ruckeri were isolated from freshwater fishes in some regions in Egypt (Mosad *et al*., 1992; Hussein *et al*., 1997; Abd El-Latif *et al*., 2001 and Ali *et al*., 2008). It is well known that diagnosis of a particular infection depends on detection and identification of its causative agent (Das *et al*., 2014). The present study was carried out to isolate and identify *Y.ruckeri* from the diseased *O. niloticus* and *C. gariepinus* and to estimate the prevalence of yersiniosis (red mouth disease) in Sohag Governorate, Upper Egypt.

MATERIALS and METHODS

Fish samples and clinical examination

Ninety-three samples of the diseased *O. niloticus* and eighty-seven samples of the diseased *C. gariepinus* were subsequently collected from the different fish farms and markets of Sohag Governorate during the period from March 2014 to March 2015. These samples included live fishes, moribund and recently dead ones. The collected fishes were transported rapidly to the laboratory in insulated ice box containing ice. They were subjected to clinical and post-mortem examination according to Amlacher (1970) for recording the clinical abnormalities present externally and internally.

Isolation of *Y. ruckeri*

Aseptically, sterile bacteriological loops from the internal organs (liver, kidney and spleen) were taken and were immediately inoculated into tryptone soya broth (TSB) and incubated under aerobic condition at 25°C for up to 48 hours. The incubated broth was streaked onto Tryptone Soya agar and incubated at

25°C for up to 48 hours (Ross *et al.*, 1966). The isolates were preserved frozen at -80°C in TSB supplemented with 15% glycerol till further use (Bastardo *et al*., 2011).

Identification on *Y. ruckeri*

This was done on basis of cultural and morphological characters, conventional biochemical tests plus API 20E system (bioMerieux, France) according to manufacturer's instructions. The isolate was identified according to criteria of Ross *et al*. (1966), Ewing *et al*. (1978), Austin and Austin (2007) and Tinsely *et al*. (2010).

RESULTS

Clinical and post-mortem examination

Examination of *O. niloticus* infected with *Y. ruckeri* revealed presence of extensive hemorrhages on the skin, congestion of the lips, isthmus region, oral cavity and the fins. Erosions of the oral cavity were detected. Internally, there were haemorrhages in the muscles, hyperemia and severe congestion of the spleen and kidney. Hemorrhagic gastroenteritis (Figs. 1, 2, 3 and 4).

Concerning *C. gariepinus* infected with *Y. ruckeri* revealed the presence of hemorrhages on the skin, lips, isthmus region & in the eyes and congestion of the fins. There were hyperemia and congestion of the spleen, kidneys, and intestine (Figs. 5, 6, 7, 8 and 9).

***Y. ruckeri* isolation from the examined fishes**

Y. ruckeri were isolated from the examined *O. niloticus* (3.2%) and *C. gariepinus* (2.3%).

Phenotypic identification of *Y. ruckeri* isolates

On Tryptone Soya agar, colonies of *Y. ruckeri* were small, round, white and creamy. Microscopical examination for Gram-stained smears prepared from these colonies, revealed Gram-ngative short bacilli to coccobacilli. They were motile Biochemical characterization of the isolates revealed that all the 5 isolates were biochemically homogeneous except in Voges-Proskauer test and gelatine liquifaction (Table 1). Based on results of phenotypic characterization for *Y. ruckeri* isolates, these isolates were belong to Biotype 1 of *Y. ruckeri* and similarity percentage of their phenotypic characteristics was ranged from 92.9 to 100%.

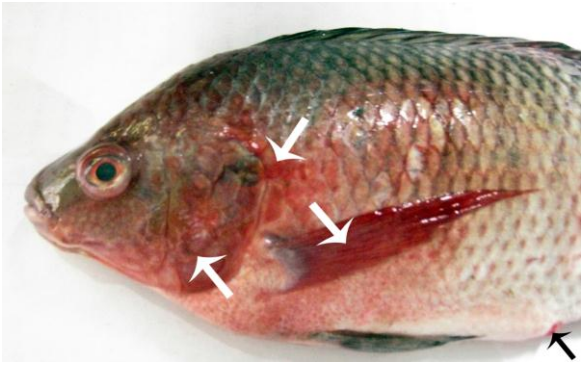

Fig. 1: *O. niloticus* infected with *Y. ruckeri* showing hemorrhages on the skin and congestion of the gills, pectoral fin and vent.

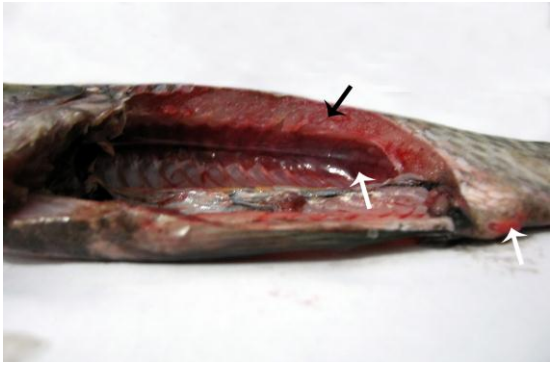

Fig. 2: *O. niloticus* infected with *Y. ruckeri* showing hemorrhages on the muscles and congestion of the kidney and vent.

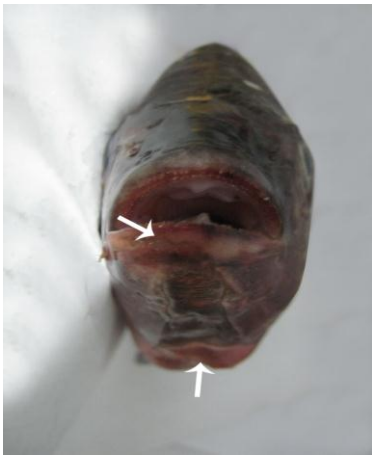

Fig. 3: *O. niloticus* infected with *Y. ruckeri* showing hemorrhages on the lips and isthmus region.

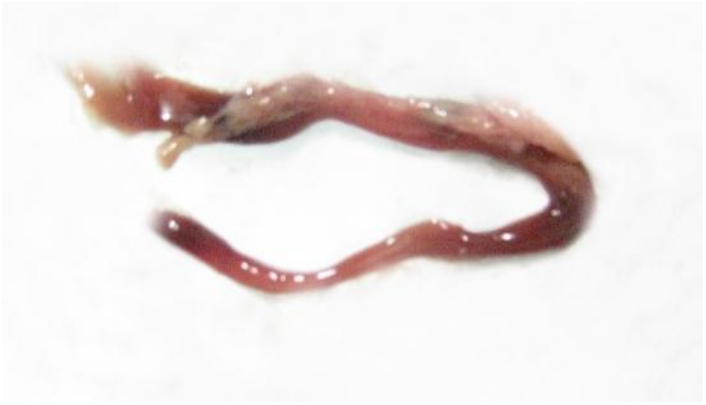

Fig. 4: Part from intestine of *O. niloticus* infected with *Y. ruckeri* showing hemorrhagic enteritis.

Fig. 5: *C. gariepinus* infected with *Y. ruckeri* showing hemorrhages on skin and congestion of pectoral fin.

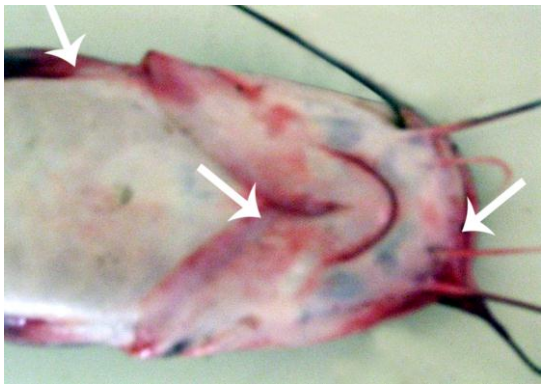

Fig. 6: *C. gariepinus* infected with *Y. ruckeri* showing hemorrhages on skin, lips and isthmus region and congestion of pectoral fins.

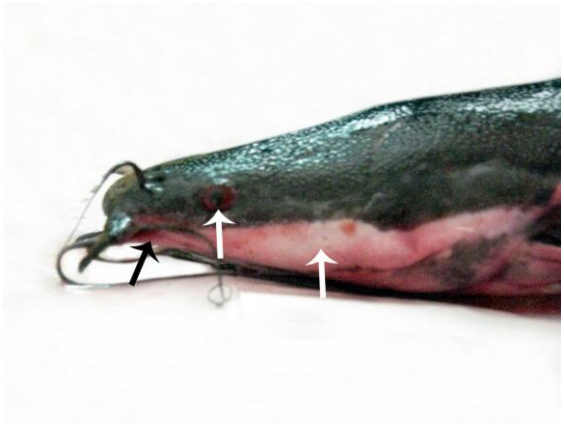

Fig. 7: *C. gariepinus* infected with *Y. ruckeri* showing hemorrhages on skin and in the eye.

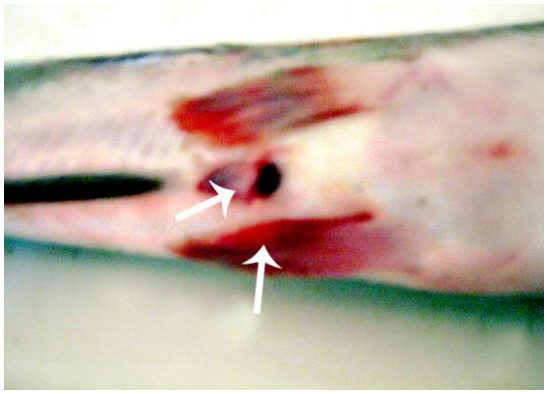

Fig. 8: *C. gariepinus* infected with *Y. ruckeri* showing congested vent and pelvic fins.

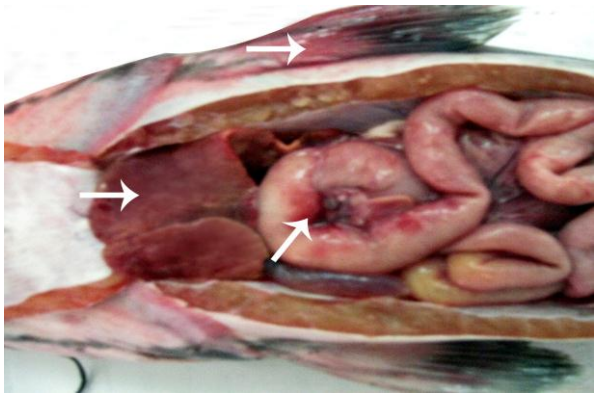

Fig. 9: *C. gariepinus* infected with *Y. ruckeri* showing congestion in the liver and intestine.

Table 1: Biochemical characteristics of *Y. ruckeri* isolates.

Test \ Isolate	Y 1	Y 2	Y 3	Y 4	Y 5
Cytochrome oxidase	-	-	-	-	-
Catalase	+	+	+	+	+
F/O of glucose	+	+	+	+	+
Fermentation of lactose	-	-	-	-	-
Methyl red reduction	+	+	+	+	+
ß-galactosidase	+	+	+	+	+
Arginine dehydrolase	-	-	-	-	-
Lysine decarboxylase	+	+	+	+	+
Ornithine decarboxylase	+	+	+	+	+
Citrate utilization	+	+	+	+	+
H_2S production	-	-	-	-	-
Urease	-	-	-	-	-
Tryptophane deaminase	-	-	-	-	-
Indole production	-	-	-	-	-
Vogus-proskauer	+	+	+	-	-
Gelatinase	+	-	+	+	+
F/O of mannitol	+	+	+	+	+
F/O of inositol	-	-	-	-	-
F/O of sorbitol	-	-	-	-	-
F/O of rhaminose	-	-	-	-	-
F/O of sucrose	-	-	-	-	-
F/O of melibiose	-	-	-	-	-
F/O of amygdaline	-	-	-	-	-
F/O of arabinose	-	-	-	-	-

DISCUSSION

Y. ruckeri causes enteric redmouth disease which is a serious bacterial septicaemia in salmonids and other fish species worldwide (Furones *et al*., 1993). In this study, it was found that prevalence of *Y. ruckeri* infection among the examined *O. niloticus* was 3.2%. This finding nearly agreed with results of Ali *et al.* (2008) who reported that incidence of *Y. ruckeri* among the diseased *O. niloticus* at Beni-Suef Governorate (2.7%). Very higher rate of *Y. ruckeri* in *O. niloticus* was recorded by Essam (2004) who isolated *Y. ruckeri* from the clinically infected, asymptomatic dead and apparently healthy *O. niloticus* from Egyptian farm with incidence 88%, 76% and 56% respectively. This disagreement may be attributed to the fluctuating climatic changes, faulty management practices and shedding of large number of bacteria in water under stress. Prevalence of *Y. ruckeri* infection among the examined *C. gariepinus* was 2.3%. Higher rate was recorded by Abd El-Latief *et al.* (2001) who recorded that prevalence of *Y. ruckeri* among the diseased *C. gariepinus* in Nile Delta region was 11.2%. This disagreement may be attributed to the fluctuating climatic changes, shedding of large number of bacteria in water under stress and location of the study.

Examination of *O. niloticus* infected with *Y. ruckeri* revealed presence of extensive hemorrhages on the skin, congestion of fins, lips, snout region & oral cavity and erosions in tissues of oral cavity. Internally, there were haemorrhages in the muscles, hyperemia and severe congestion of the spleen, kidneys, and intestine (hemorrhagic gastroenteritis). These clinical signs and post-mortem lesions were similar to those reported by El-Gamal *et al.* (2005)

and Eissa *et al*. (2008). While examination of *C. gariepinus* infected with *Y. ruckeri* pointed number of the clinical abnormalities including hemorrhages on the skin, lips, snout region & in the eyes and congestion of the fins. Internally, there were hyperemia and congestion of the spleen, kidneys, and intestine. Similar signs and post-mortem lesions were reported by Abd El-Latief *et al.* (2001). The distinctive redmouth feature of ERM was recorded in some affected *C. gariepinus* and this agreed with Horne and Barnes (1999) who stated that the distinctive redmouth feature of ERM isn't invariably noticed.

Y. ruckeri suspected colonies on Tryptone Soya agar grown forming small, round, white-creamy and this result was similar to findings of Ross *et al*. (1966), Seker *et al*. (2011) and Seker *et al*. (2012). By microscopical examination, the isolates were Gram-negative short bacilli to coccobacilli and motile. These results were similar to findings of Abd El-Latif *et al*. (2001) who isolated Gram-negative, motile short coccobacilli or short-medium sized bacilli *Y. ruckeri* from *O. niloticus, C. lazera* and *C. carpio* and to some extent differed from findings of Bastardo *et al*. (2011) who isolated five non-motile *Y. ruckeri* strains in Peru.

In regarding to the biochemical characteristics of *Y. ruckeri* isolates, results of the conventional biochemical tests and API 20E assay as shown in table (1) revealed that all *Y. ruckeri* isolates were biochemically homogeneous except in Voges-Proskauer and gelatine liquifaction tests. All the isolates were positive in lysine decarboxylase, ornithine decarboxylase, β-Galactosidase, citrate utilization, methyl red and utilization of glucose and mannitol and negative in indole production, H_2S production, urease, arginine dihydrolase, tryptophane deaminase, and utilization of inositol, sorbitol, rhaminose, sucrose, melibiose, amygdalin and arabinose while 60% and 80% of them were positive in Voges-Proskauer test and gelatine liquifaction test respectively. The biochemical characteristics of *Y. ruckeri* in this study were in agreement with findings of Ewing *et al.* (1978).

The variation among *Y. ruckeri* isolates in Voges-Proskauer test was reported by Abd El-Latif *et al*. (2001) and Bastardo *et al*. (2011) who found that 10.4% and 23.4% of *Y. ruckeri* isolates were positive in this test respectively. In contrast, Eissa *et al*. (2008) and Joh *et al*. (2010) found that *Y. ruckeri* isolates were homogenous and Voges-Proskauer positive while Şeker *et al*. (2012) and Altun *et al*. (2013) found that all *Y. ruckeri* isolates were Voges-Proskauer negative. Also, the variation among *Y. ruckeri* isolates in gelatine liquifaction test was reported by Altun *et al*. (2010) who reported that 23.8% of *Y. ruckeri* isolates were gelatine liquifaction test negative while Eissa *et al*. (2008) reported that all *Y. ruckeri* isolates were gelatine liquifaction test positive. The variation in any phenotypic characteristic may be attributed to presence or absence of plasmid (s) that controls its metabolic trait.

Based on results of the phenotypic characterization of *Y. ruckeri* isolates, it was concluded that the isolates were homogenous except in Voges-Proskauer and gelatine liquifaction tests, similarity of their phenotypic characteristics was ranged from 92.9 to 100% as showed in table (19) and they were belonged to Biotype 1.

CONCLUSION

In this study, five *Y. ruckeri* isolates were isolated from *O. niloticus* and *C. gariepinus* at Sohag Governorate for the first time at this region, these isolates were homogeneous in their phenotypic characters except in Voges-Proskauer and gelatine liquifaction tests.

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مرض اليرسينيوزس فى أسماك المياه العذبه بمحافظة سوهاج بصعيد مصر

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تسبب اليرسينيا روكرى مرض الفم الأحمر المعوى أو اليرسينيوزس وهو مرض بكتيرى خطير لتسمم دم الأسماك البحرية وأسماك المياه العذبة. أجريت هذه الدراسة لفحص مدى إنتشار اليرسينيا روكرى فى أسماك البلطي النيلي والأسماك القطية (القراميط) في محافظة سوهاج و للتوصيف الشكلى للعزلات من هذه الأسماك. لذلك فقد تم تجميع ٩٣ عينة من أسماك البلطى النيلى المريضة و ٨٧ عينة من الأسماك القطية (القراميط) المريضة من مناطق مختلفة في محافظة سوهاج خلال الفترة من مارس 2014 وحتى مارس 2015 . وقد خضعت هذه العينات لفحص العلامات السريرية والتشريحية ثم للفحص البكتريولوجي للكبد والكلى والطحال و تم فحص الصفات المورفولوجية والبيوكيميائية للعزلات ببعض الطرق التقليدية ونظام API 20E . كانت نتائج الفحص البكتريولوجي للكبد و الكلى و الطحال لهذه العينات هى عزل ٥ عترات من اليرسينيا روكرى [٣ عترات من أسماك البلطى النيلى بنسبة 3.2% و2 عترة من الأسماك القطية (القراميط) بنسبة 2.3%]. كما كشف فحص الصفات المورفولوجية والبيوكيميائية لعزلات اليرسينيا روكرى أن جميع العزلات متماثلة إلا في إختبار الفوجس بروسكاور وإسالة الجيلاتين وأن نسبة التشابه بينهم تراوحت بين ٩٢.٩ و١٠٠% كما أنها تنتمى للنوع الحيوي الأول من اليرسينيا روكرى.