Available Online at http://www.recentscientific.com

ISSN: 0976-3031

International Journal of Recent Scientific Research
Vol. 6, Issue, 6, pp.4374-4377, June, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

DETECTION OF THE CAUSATIVE AGENTS OF BACTERIAL FISH SEPTICEMIA OF TILAPIA AND CLARAIS IN KHARTOUM STATE

Maimona[1], Samia[2] and Sabiel[3], Y. A

[1]Ministry of Animal Resources, Fisheries and Pasture, Department of Epidemics
[2]Sudan University of Science and Technology, College of Science and Animal Production, Khartoum, Sudan
[3]Veterinary Research Institute, Khartoum, Sudan

ARTICLE INFO

Article History:

Received 5th, May, 2015
Received in revised form 12th, May, 2015
Accepted 6th, June, 2015
Published online 28th, June, 2015

Key words:

Tilapia, Clarias, Aeromonas spp., septicaemia

ABSTRACT

This a study was conducted in Khartoum State, Sudan to detect the causative agents of bacterial fish septicaemia (*Aeromonas spp.* and *Pseudomonas spp.*) and their prevalence in three seasons of years (Winter, Summer and Autumn).

A total of 35 fish samples ;19 *Oreochromis niloticus* and 16 *Clarias sp*., were collected from Jabal Awalia dam and Elshagera localities (river Nile) and from Elshagara Fish Research Center farm in the period from December 2012 to June 2013. The samples were taken from gills, skin, intestine, liver, stomach and kidney. The experiment was conducted in the institute of Veterinary Research Institute, Bacteriology Department, Suba, Khartoum State.

Different methods of identification were used for the identification of the isolates including conventional methods and rapid identification system by using API 20NE kits Strips.

Statistical analysis of the obtained results revealed high significant differences between the seasons of the year ($P < 0.01$), different organs ($P < 0.01$) and significant differences between *Oreochromis niloticus* and *Clarias* sp. ($P < 0.05 > 0.01$) in the abundance of *Aeromonas spp*.

The results also viewed that *Pseudomonas spp*. were not detected in all samples investigated in two species in all seasons throughout this study.

INTRODUCTION

Today, the global community faces multiple and interlinked challenges ranging from the impact of the ongoing financial and economic crisis to greater climate change vulnerabilities and extreme weather events. Fish and fishery products represent a very valuable source of protein and essential micronutrients for balanced nutrition and good health (Arni, 2012). The fisheries resources of Sudan include both inland and marine fisheries. The Red Sea, with a coastline of approximately 480 km, forms the marine fisheries resources. The inland fisheries resources, on the other hand, are centered in the White Nile, the Blue Nile, the main River Nile and their tributaries. Also there are several big lakes formed by the construction of dams. The first Demonstration Fish Farm was set up at Elshagara, six miles south of Khartoum on the White Nile, "to study problems regarding fish culture in the Sudan and to investigate its application to autochthonous and also, perhaps to other species" (Anon, 1955).

Bacterial diseases are responsible for heavy mortality in both wild and cultured fish. The actual role of these microorganisms may vary from that of a primary pathogen to that of an opportunist invader of a host rendered moribund by some other disease process (Richards and Roberts, 1978). External conditions, especially temperature, appears to influence greatly on the nature of the infection (Bisset, 1947). The characteristic symptoms of the disease produced by the bacteria is a remarkable septicemia, hemorrhage in the skin of the mouth region, opercula and ventral side of the body (Wakabayashi and Egusa, 1972). The clinical signs and P.M lesions were absence of reflexes, dark discoloration, and scale loss, fin rot hemorrhage of the body and congestion of the internal organs. These signs have been reported by Mushiake *et al*., (1984). Many of these organisms are a usual component of the bacterial flora in aquatic habitats, particularly atrophic systems. Stressors, often inevitable in most culture systems, predispose fish to bacterial-borne diseases (Snieszko, 1974). The course of events from stress to predisposition to infection, include physiological changes (described as a general alarm response syndrome) the consequences of which are to enforce barriers, normally preventing entry of bacteria to fish inner systems and at the same time incapacitating fish defense responses and immune reactions (Mazeaud *et al*., 1977); Barton and Iwama, 1991).

Corresponding author:* **Maimona
Ministry of Animal Resources, Fisheries and Pasture, Department of Epidemics

MATERIALS AND METHODS

A total of 19 fish samples of *Oreochromis niloticus* ranging between 15to 25 cm. in length and 16 samples of *Clarias lazera* range between 15 to 55 cm. in length were collected from three different localities in Khartoum State; Jabal Awlia Dam, Elshgagara capture, and Research of Fish Center pond during December 2012 to June 2013.Immediately after catching the body surfaces of the fish was examined for the presence of lesions such as ulcers, furuncles and granuloma especially fins, tail and gill were examined for proliferative and necrotic changes. Each fish was placed in sterile plastic bag then placed in a thermo flask containing ice and transported to the Department of Bacteriology, Veterinary Research Institute for further examinations. Swab sample was taken from the body surface of each fish followed by the examination of the internal organs for pathological lesions. Approximately one gram from Gill, liver, intestine, kidney, stomach and skin were removed aseptically and homogenized separately. Sterile cotton swabs were dipped into each suspension and streaked onto two blood agar plates, one plate was incubated at 37ºC and the other at 35ºC for 24hours. The obtained colonies were further subculture and incubated further for 24 hours .The pure cultures were preserved at 4ºC for subsequent identification.

Gram negative isolate were identified by standard bacteriological methods according to Barrow and Felltham, 1993 using primary tests and secondary biochemical tests. Primary tests included; gram reaction, motility, aerobic and anaerobic growth, catalase, oxidase, acid from glucose and oxidation fermentation test.

Isolates that suspected to be belonging to the genera Aeromonas or Pseudomonas were further tested by Api 20 NE strips which are standardized system for the identification of non – fastidious, non – enteric gram–negative rods and was performed according to the manufacture´s instruction (Biomerieux, France, 2012). Briefly 3-4 colonies for a fresh culture of the organism to be tested were picked and emulsified in 5ml medium. The suspension was adjusted to McFarland tube No. 0.5 and with the sterile Pasteur pipette the strip of the Api20 NE was inoculated with the suspension from the test NO_3 to the test PNPG.300µL of the remaining suspension was transferred to the Api AUX medium, shaken well the inoculated to the tests from GLU to PAC. Sterile paraffin oil was over laid over the tests GLU, ADH, and URE. The inoculated strip was placed into a wet incubation box and incubated at 37C .The first reading of the tests was done after 24 hours and the final reading after 48 hours. The result was recorded in the result sheet provided by the manufacturer and interpreted by the Api Lab Plus soft ware.

Total viable count (TVC)

The test was performed according to Quinn *et al*, (1999).Ten folded serial dilutions of the original samples were prepared in sterile test tubes. An inoculums of 0.1 ml of each dilution was placed on the surface of plate count agar, then spread evenly over the entire surface of a plate count agar (two plates for each dilution) using glass rod then incubated for 24 hours at 37ºC. Plates that yielded counts between 30and 300 were used for calculating the total viable count.

Statistical analysis

The obtained results were analyzed statistically using analysis of variance

(ANOVA). Duncan's multiple range test (Duncan, 1995) was used to evaluate the mean differences among different treatment at the 0.05 significant levels.

RESULTS

Bacterial isolates recovered were identified according to Barrow and Felltham (1993). Most bacteria isolated from the samples were dominantly gram-negative rods and bacterial genera were identified from all samples in all seasons were *Aeromonas spp***.**

Out of the 173 examined fish samples, 179(27.76%) showed no growth and 94(44.36%) showed growth of different types of bacteria. Twentysix (27.76%) of the isolates were *Aeromonas* spp. while no growth of *Pseudomonas spp*. was observed in all cultured samples. The results showed that the prevalence of *Aeromonas* spp.

In Tilapia fish was 8(8.7%) while in Clarias 18(24.6%) out of the isolated organisms. It is also showed that the isolation rates of *Aeromonas* spp. from the different organs of the Clarais fish was 6(33.33%) from the intestine, 4(22.22%) Gills, 3(16.76%) skin, 2(11.11%) from both stomach and kidneys and 1(5.56%) from intestine, while for Tilapia fish the prevalence rates were 3(37.5%), 2(25%), 1(12.5) and zero% from intestine, Gills, skin, liver and stomach respectively. The results also showed that the isolation rate of Aeromonas from intestines was higher in both Tilapia and Clarias fish (Table 1).

In addition, the results obtained revealed that three *Aeromonas spp*. were isolated and identified by the use of Api 20 NE strips (*A. hydrophylia*, *A. sobria* and *A. salmonicia*. Moreover, the isolation rate of *Aeromonas spp*. from the both fish in different season had showed no significant differences (Table 2). The results obtained also revealed that there is high prevalence of *Aeromonas spp*. in Autumn then in Summer and least prevalence observed in Winter as in table 2

Table 1 Frequency of isolation of Aeromonas spp. from Tilapia and Clarias fish in different organs

Organ	No. of positive samples of Aeromonas spp.		Number of Aeromonas spp. isolated from Tilapia and Claris
	Tilapia	Claris	
Gills	2(25%)	4(22.22%)	6(23.1%)
Skin	2(25%)	3(16.67%)	5(19.2%)
Stomach	0 (0.0%)	2(11.11%)	2(7.7%)
Liver	1(12.5%)	1(5.56%)	2(7.7%)
Kidney	0 (0.0%)	2(11.11%)	2(7.7%)
Intestine	3(37.5%)	6(33.33%)	9(34.6%)
Total	8(100%)	18(100%)	26(100%)

Table 2 Frequency of isolation of Aeromonas spp. from Tilapia and Claris fish in different seasons

Season	No. of fish examined		No. of no growth samples		No. of isolated Aeromonas		No. of other isolated organisms		Total isolated organisms	% of Aeromonas spp.
	Tilapia	Claris	Tilapia	Claris	Tilapia	Claris	Tilapia	Claris		
Winter	9	6	19	3	2	3	17	10	32	5(15.6%)
Summer	3	5	5	12	2	5	11	12	30	7(23.3%)
Autumn	7	5	35	5	4	10	3	15	42	14(33.3%)
Total	19	16	59	20	8	18	31	37	94	26(100%)

DISCUSSION

Fish diseases due to bacterial infections are the major problems in wild and aquaculture as it found naturally in the fish environment and under certain stress condition causes severe economic losses to fish (Olsson *et al*., 1998). Kaneko (1971) reported that the bacterial flora on fish reflected the biology of aquatic environment. Nowotny (1979) reported that the nature of pathogenesis caused by all gram-negative bacteria was almost similar and the disease process caused by bacterial toxins.

The present study, erected that all *Aeromonas spp*. found were motile and catalase , oxidase, NaCl 6%, were positive, Nitrate reduced to Nitrite and sugars are attacked fermentative and gas may be produced, this agree with finding by (Toranzo *et al*.,1989).

Among all other bacteria, Aeromonas and Pseudomonas are the major bacterial fish pathogens which are widely distributed in aquatic organisms in nature (Islam, 1996).

In the present study; *Aeromonas spp*. were isolated from Tilapia and

Clarias. These finding is agree with the finding of Ortega *et al*., 1996.

The study revealed that the bacterial load was high in Autumn, one of the reasons possibly being that the high ambient temperature in the water body was close to optimum for many mesophilic bacteria in natural systems (Rheinheimer, 1985), and some workers suggested that the bacterial load in fish might be increased with the increase of water temperature (Fernandes *et al*., 1997; Hossain *et al*., 1999). Also this result disagree with the finding of Rekhari *et al*., 2014 and Abd-Elall *et al*., 2014 who found that the bacterial load is higher in summer season in cultured fish.

In the present study; *Aeromonas spp*. were isolated from different organs of the fish such as intestine, gills, skin, liver, stomach and kidney with isolation percentage 37.5%, 25%,25%,12.5%,0% and 0% respectively. A similar finding obtained by authors: Surendran, and Gopakumar (1982), Sersy *et al*.,(1996), Spanggard and Huber, (2000).

Generally, the concentration of bacteria in different fish organs depending on fish species (Sersy *et al*., 1996).

Also Beri *et al*., (1989) noticed that the different proportion of bacterial profile was due to the seasonal variation, generic difference and physiological activity of the fishes.

Szczuka and Kaznowski (2004) indicated that these bacteria are widely spread in the environment, especially in surface water and sewage. The presence of different *Aeromonas* species indicates increasing pollution.

In present study, three species of genus Aeromonas were isolated (*Aeromonas hydrophyla*, *Aeromonas sobria* and *Aeromonas salmonicida*) by uses API 20 NE system in last group of samples. Also this result revealed that *Pseudomonas spp*. were not isolated during this study. But in the previous study noted that considerable numbers of *Pseudomonas spp*. were found only in winter and autumn, (Chowdhury *et al*., 1989), and predominant in water, sediment, skin and intestine of culture of common carp (Rekhari *et al*., 2014). Also Golchin *et al*., 2014 used API2OE as diagnostic system to identify the bacteria that cause fin rot and the result showed that Aeromonas genus with 10% frequency while Pseudomonas genus with 25% frequency.

Hence, this is an endeavor for the quantities and qualitative estimation of the bacteria present in the fish in order to assess the quality and health status of fish.

References

Abd- Elall, A. M. and Abd- El- Kader, M.A. and Atia, A., (2014). Occurance , seasonal variation and virulence of Aeromonas hydrophila and A. caviae in fish farms at East Delta, Egypt. Global vetrrnarian 13 (3):328- 336.

Anon, A., 1955. The Sudan Today.Social and Economic Progress. Ministry of Social Affairs, Sudan Government and Pekkola, (1918) Seasonal Occurrence and Edibility of fish at Khartoum.1918,Sudan Notes and Records,(1):88 – 98.

Arni, M. M., (2012).The state of world fisheries and aquaculture. Publishing Policy and Support Branch.Office of Knowledge Exchange, Research and Extension.FAO, Viale delle Terme di Caracalla 00153 Rome, Italy.

Barrow, G. I. and Feltham, R. K. A., (1993).*Cowan and Steel's Manual* for the Identification of Medical Bacteria. 3rd ed. Cambridge University Pres. Cambridge.

Barton, B. A. & Iwama, G. K., (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Rev. Fish Dis., 1: 3–26.

Beri, H. k., James, M. A., and Solanki, K. K., (1989). Bacterial flora of some fishes in *Maharashtra saurashtra* coast (India), *Jour.Fis.Dis.Sci.Technol.*, 26(6) 318 -321.

Bisset, K. A., (1946). The effect of temperature on non specific infections of fish. *J. Path. Bact*. 58, 251.

Chowdhury, M. B. Muniruzzaman, M. Uddin, M. N., (1989). Study on the intestinal bacterial flora of tilapia *Oreochromis niloticus*. *Bangladesh J. Aquac.* 11, 65–70.

Duncan, D.B. (1995). Muliblerange and multiole (F) test. Biometrics, 11: 1- 42.

Fernandes, C. F., Flick, G. J. Silva, J. L. McCasky, T. A., (1997). Influence of processing schemes on indicative bacteria and quality of fresh aquacultured catfish fillets. *J. Food Prot.* 60, 54–58.

Golchin, A. M. and Assareh, A., (2014). Bacterial study of fin rot in broun trout by API20E. *Pakistan journal of biological science* 17 (3):434- 438.

Hossain, M. M., Uddin, M. N., Islam, M. N., Chakraborty, S. C., Kamal, M., (1999). Study on the intestinal bacteria of Labeo rohita (Ham.). *Bangladesh J. Fish. Res*. 3, 63–66.

Islam, M . S., (1996). studies on the bacteria *Pseudomonas spp*. In farmed fish and water.in and around Mymensigh . M.S. Thesis. Department of Fisheries Biology and Limnology, Faculty of Fisheries, Bangaladesh Agricultural Uiversity, Mymensigh, Bangaladesh 101 pp.

Kaneko, S. (1971). Microbiological study of fresh fish. New Food Industry 13: 76- 80.

Mazeaud, M. M., Maseaud, F. and Donaldson, E.M., (1977). Primary and secondary effects of stress in fish: some new data with general review. Trans. Am. Fish. Soc., 106: 201–212.

Mushiake, K., Muroga, K. and Nakai, T. (1984). Increased susceptibility of Japanese eel *Anguilla japonica* to *Edwardsiella tarda* and *Pseudomonas anguilliseptica* following exposure to copper. Bulletin of the Japanese Society of Scientific Fisheries 50, 1797-1801.

Nowotny, A., (1979). Molecular aspects of endotoxins. Bacterial, Rev.,33- 72.

Olsson, J. C., Joborn, Westerdahl, A.; Blomberg, L., Kjelleberg, S. and Conway, P. L., (1998). Survival, persistence and proliferation of *Vibrio anguillarum* in Juvenile turbot, *Scophthalmus maximus* (L.), intestine and faeces. *Journal of Fish Diseases*, 21: 1-9.

Ortega, C. Muzquiz, J. Fernandez, A. and De Blas, I., (1996). Water quality parameters associate with Aeromonas spp. affected hatcheries. Veterinary Research, BioMed control, 27 (6): 553-560.

Rekhari, Y. C., Agrawal, R., Trakroo, M. and Tiwari, H., (2014).Qualitative and Quanative study on bacterial flora of farm raised common carp (*Cyprinus carpio*) in India. *African Journal of Microbial research* 8 (11): 1125-1129.

Rheinheimer, G., (1985). Aquatic Microbiology, 3rd ed. University of Kiel, West Germany. Wiley, Chichester, New York, Brisbane, Toronto, p. 257.

Richards, R. H. and Roberts, R. J. (1978). The Bacterial Diseases. In: J.F.N. Abowei, and O.F. Briyai, (2011). A Review of Some Bacteria Diseases in Africa Culture Fisheries. *Asian Journal of Medical Sciences* 3(5): 206-217, 2011.

Sersy, A . Nermeen, M. A. Toshiko, U. and shiro, H., (1996). Occourence of Vibrionaceae bacterial in five kind species of marine fish organs in Souther area of Japbn.No. 29(1996) 129 – 138.

Snieszko, S. F., (1974). The effect of environmental stress on outbreaks of infectious diseases of fishes. *J. Fish Biol.*, 6: 197–208.

Spanggard, B., Huber, I., (2000). The microflora of rainbow trout intestine. Acompairson of traditional and molecular identification, Science Direct – Aquaculture, 182 (2000) 1 – 15.

Surendran, P. K. and Layer, K. M., (1984). The bacterial flora of pearl spot, Etroplus suratensis caught from Cochin backwater, Proc. Symp. Coastal aquaculture. Cochin. 3(1984) 852 -855.

Szczuka, E. and Kaznowski, A., (2004). In: Hala Mohamed Rifaat2007. Bacterial Quality of River Nile Water at Cairo Region in Egypt. Suoseura-Finnish Peatland Society Suo 59 (1–2): 1–8.

Toranzo, A. E., Baya, A. M., Romalde, J. J. and Hetrick, F. M., (1989). Association of *Aeromonas sobria* with mortahties of adult gizzard shad, Dorosoma cepedianum Lesueur. *Journal of Fish Diseases* 12, 439-448.

Wakabayashi, H. and Egusa, S. (1972). Characteristics *of a Pseudomonas* sp. from an epizootic of pond-cultured eels *(Anguilla japonica*). Bulletin of the Japanese Society of Scientific Fisheries 38 Quinn, P.J., Cartner, M. E., Makey, B.K. and Cartner, G. R. (1999). Clinical Vetrinary Microbiology. Virginia U.S.A.

How to cite this article:

Maimona *et al*., Detection of the Causative Agents of Bacterial Fish Septicemia of Tilapia and Clarais in Khartoum State. *International Journal of Recent Scientific Research Vol. 6, Issue, 6, pp.4374-4377, June, 2015*

****