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COLUMNARIS DISEASE IN SHARPTOOTH CATFISH, *CLARIAS GARIEPINUS*

(With 5 Tables and 2 Figures)

By

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مرض الكولمنارس فى الأسماك القطية النيلية (القراميط)

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الهدف من هذه البحث هو اجراء دراسة ميدانية لتسجيل إصابة الأسماك القطية النيلية (القراميط) بمرض الكولمنارس وكذلك التغيرات المرضية نتيجة الإصابة. تم فحص عدد ١٤٤ سمكة على مدار عام بمعدل ١٢ أسماك شهريا ذات الأوزان المختلفة والتى تم جمعها بشكل عشوائي من روافد نهر النيل المختلفة بمحافظه قنا كشف الفحص الظاهري، البكتريولوجى بان نسبه الاصابه بمرض الكولمنارس ٤,٨٦%. كما تم مقارنة معدل الإصابة للأوزان المختلفة وفى فصول السنة المختلفة. الأعراض الظاهرية التي شوهدت على خياشيم الأسماك المصابة هى ظهور الخياشيم باللون الشاحب وفى بعض الأسماك شوهد زيادة في نمو طبقات الخياشيم بينما في البعض الآخر شوهد تهتك في طبقات الخياشيم.أيضا شوهد وجود تقرحات على الجلد في المنطقة تحت الزعنفة الزيليه مع تآكل في الزعنفة الزيليه في بعض من الأسماك المفحوصة. الأعراض الظاهرية لأسماك القرموط المصابة تجريبيا بعتره الفلافوبكتريم كولمنارس توضح انه يوجد التهابات في الجلد، تقصف في الزعانف والخياشيم شاحبه في اللون مع تهتك في طبقات الخياشيم الذي يبدا من الخارج. أيضا توجد منطقه باهته عند قاعدة الزعنفة الظهرية تسمى اصابه السدل التي تبدأ صغيره ثم تصبح كبيره. الاصابه بالفلافوبكتريم كولمنارس تنتقل تجريبيا بواسطة الغمر مع جرح أو خدش السمك سواء الخياشيم أو الجلد عند قاعدة الزعنفة الظهرية. بينما العدوى بواسطة الغمر فقط فشلت في ظهور المرض تجريبيا. توضح نتيجة عمل اختبار حساسية دوائية لميكروب الفلافوبكتريم كلومينارس هى انه حساس جدا الى السيبروفلوكساسين، تارفيد (اوفلوكساسين) والسيفوتاكزيم (كلافوران)، ولكن مقاوم الى السيفرادين (فيلوسيف).

SUMMARY

The aim of this study was to investigate the prevalence of columnaris disease in wild sharptooth catfish, *Clarias gariepinus*, in Upper Egypt. Columnaris was detected in 7 (4.86%) fish out of the 144 fish collected indicating light infection. The main signs observed on fish were paleness and sloughing of gill filaments, in addition to skin erosions and fin rot that were seen on some specimens. No specific pattern was detected in weight susceptibility of sharptooth catfish to columnaris. Prevalence of the disease was highest in autumn than in other seasons of the year. Pathogenicity of *Flavobacterium columnare* isolated in the present study was investigated through an immersion challenge. Fish groups to be challenged were either subjected to skin or gill scarification or remained un-scarified. All challenged fish were immersed in $3.5X10^7$ colony forming units/ml of *F. columnare* challenge suspension. Clinical signs as loss of appetite and sluggish movement began to appear on fish 48 hours post challenge, while respiratory manifestations and skin erosions appeared later. Two fish died out of the group that had skin scarification. It was interesting to notice that fish challenged through immersion without scarification did not develop typical signs of infection. The antibiogram of *F. columnare* was also investigated where it was highly sensitive to cefotaxim, ciprofloxacin and ofloxacin, but resistant to cephradine, while moderately to less sensitive to trimethoprim-sulfamethoxazole, E-moxclav, colistin, and flummox.

Key words: *Columnaris, sharptooth catfish, flavobacterium, saddle-back, gill rot*

INTRODUCTION

Columnaris disease is one of the oldest known bacterial diseases of warm water fish. This disease was first described in 1922 as the cause of a fish kill in The Mississippi River, USA. The causative agent was named *Bacillus columnaris* because wet mounts prepared from diseased fish showed characteristic column-like masses of bacterial cells (Davis 1922). The nomenclature of the bacterium has changed many times and included the synonyms *Chondrococcus columnaris*, *Cytophaga columnaris* and *Flexibacter columnaris* (Bernardet and Grimont 1989), but in 1996 it was transferred to the genus *Flavobacterium* and named *Flavobacterium columnare* (Bernardet *et al.*, 1996).

Columnaris is distributed world wide in aquatic environments, affecting wild and cultured fish as well as ornamental fish, and may result in acute or chronic infections in both coldwater and warm water fishes (Austin and Austin 1999). It occurs both as external or systemic infections that result in significant losses of fish, particularly at warm summer temperatures (Pacha and Ordal 1970 and Becker and Fujihara 1978). Columnaris disease is enhanced by stress factors such as unfavorable water temperature, crowding, injury, another disease, or improper fish husbandry.

Columnaris disease usually begins as an external infection of fins, body surface or gills (Austin and Austin 1999). In temperate fish, columnaris disease is recognized by the appearance of grayish white or yellow areas of erosion, usually surrounded by a reddish hyperemic zone on the body surfaces or the gills of fish. When these lesions occur around the dorsal fin, they are called saddle-back lesions, which are typical for columnaris disease (Austin and Austin, 1987).

The ubiquitous distribution of the organism in fresh water environments and the tendency for fish to acquire the disease after mechanical and/or environmental stress makes *F. columnare* among the most detected pathogens in cultured, ornamental, and wild fish populations (Shamsudin, 1994 and Shotts and Starliper, 1999). There are few literature in hand about columnaris in Egypt. Thus, the aim of this study was to investigate the prevalence of columnaris disease in wild sharptooth catfish *Clarias gariepinus*, in Upper Egypt and detect the pathogenicity of isolated *F. columnare* strains to apparently healthy fish. Also, weight and seasonal susceptibility to the disease were investigated. This study is a component of a comprehensive research project on all *Flavobacterium spp.* in sharptooth catfish.

MATERIALS and METHODS

Fish collectio:

A total of 144 freshly caught sharptooth catfish, *Clarias gariepinus*, were collected from different localities of The River Nile, Qena Governorate, over a calendar year (12 fish/month). Collected fish were divided according to their body weight into three groups (Table 1). The body weight of examined fish ranged from 100 to 500g with mean length of 27.7 to 37.5cm. Fish were transported to The Aquatic Animals Diagnostic Laboratory, Faculty of Veterinary Medicine, South Valley University, Qena, where clinical and bacteriological examination had been conducted.

Table 1: *Clarias gariepinus* groups according to body weight and length

No. of fish	Weight group	Mean length
48	100-200g	27.7cm
48	201-300g	33.8cm
48	301-500g	37.5cm

Clinical and Bacteriological Examination of fish:

Fish were examined for clinical signs or external lesions according to Stoskopf (1993). Opercula were removed to expose the gill tissues and samples were cultured into cytophaga agar medium (Anacker and Ordal, 1959) and incubated at 25° ± 2°C for 48 hours. Samples from fins were also, cultured directly on cytophaga agar medium. Suspected colonies were picked up and subcultured for purification and identification according to Austin and Austin (1987).

Identification of bacterial isolates:

Bacterial isolates were identified based on colony morphology and ability to produce flexirubin pigment, growth on selective media, microscopic examination (Gram stain and motility test), biochemical characters that include oxidase, catalase, ability to produce H_2S, methyl red, and sugar fermentation ability (glucose, maltose, and sucrose) according to Austin and Austin (1987).

Pathogenicity of *Flavobacterium columnare* to *Clarias gariepinus*:

Fish: Apparently healthy sharptooth catfish with an average body weight of 150-200g and mean length of 27.7-28.8cm were obtained from the River Nile. Fish were acclimated to laboratory conditions for two weeks according to the protocol of maintaining bioassay fish described by Ellsaesser and Clem (1986).

Bacterial challenge suspension and counts: A preliminary growth curve study was conducted to determine counts of colony forming units (cfu) of *F. columnare* in cytophaga broth at various growth phases using standard plate count method (Elkamel *et al.*, 2003). *F. columnare* was grown in cytophaga broth at 25°C to reach an optical density of 0.3 at 600nm, which was found to be equivalent to $7.0X10^7$cfu/ml. Challenge suspension was prepared by diluting the above *F. columnare* culture in sterile water (1:1) to reach $3.5X10^7$cfu/ml.

Experimental infection: Acclimated sharptooth catfish were divided into groups of 7 fish each. Fish groups to be challenged were either subjected to skin or gill scarification (Fish and Rucker 1943) or remained un-scarified. All challenged fish were immersed for 30 min in the challenge suspension prepared, while the control group was un-

scarified and immersed in a solution of sterile cytophaga broth and sterile water (1:1) as shown in Table 2. Clinical signs and mortalities were recorded daily over 21 days. Moribund fish were examined to record clinical signs and isolate the bacteria from lesion sites.

Table 2: Experimental infection of sharptooth catfish, *Clarias gariepinus*, with *Flavobacterium columnare*.

Group	No. of fish	Route	Immersion dose
Gill scarified	7	Gill scarification	$3.5X10^7$ cfu/ml
Skin scarified	7	Skin scarification	$3.5X10^7$ cfu/ml
Un-scarified	7	-	$3.5X10^7$ cfu/ml
Un-scarified control	7	-	Sterile broth and water

Antibiogram:

Antimicrobial susceptibility test was investigated on cytophaga agar medium against 8 antimicrobial agents using the disc diffusion technique as described by Finegold and Martin (1982). Antibiotic sensitivity was determined based on the diameter of clearance zone around the discs. Tested antimicrobial agents were cefotaxim (30 µg), cephradine (30 µg), Ciprofloxacin (5 µg), colistin (25 µg), E-moxclav (30 µg), flumox (10 µg), Ofloxacin (10 µg) and Trimethoprim-sulfamethoxazole (25 µg).

RESULTS

Clinical Examination:

Examined wild fish showed a wide variety of gill and skin lesions. Nearly one third of the fish had pale gills, while few showed sloughing of gill filaments (Fig. 1) or hyperplasia in gills. On the other hand, some fish had erosions at the caudal peduncle and fin while others had caudal fin rot.

Bacteriological isolation and identification:

Bacteriological examination of the collected fish resulted in isolation of 59 isolates. According to morphological and cultural characters, 34 isolates were suspected to be *Flavobacterium species*. According to biochemical characters, however, the isolates were identified as *F. columnare* (7 isolates), *F. psychrophilum* (21isoaltes) and *F. branchiophilum* (6 isolates). *F. columnare* colonies were mucoid, yellow or orange in colour with irregular edges and showed gliding movement on cytophaga agar media.

Table 3: Phenotypic characteristics of suspected *Flavobacterium columnare* isolates.

Biochemical reactions	*Flavobacterium columnare*
Gram stain	-ve
Flexirubin pigment	4 / 3
Gliding motility	+
Oxidase	–
Catalase	+
H_2S production	+
Glucose	–
Sucrose	–
Maltose	–
Methyl red	–
Growth at 37°C after 24 hours	+

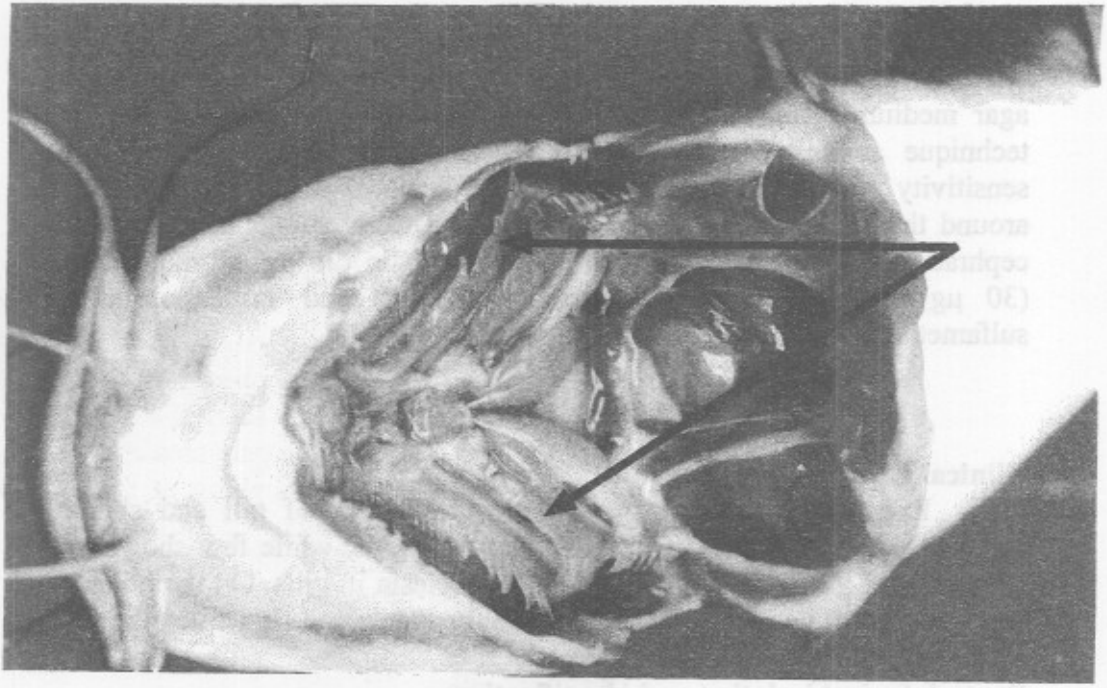

Fig. 1: Sharptooth catfish, *Clarias gariepinus*, naturally infected with *Flavobacterium columnare* showing paleness and sloughing of gill filaments (arrows).

Survey and prevalence:

Body weight and seasonal susceptibility of sharptooth catfish to *F. columnare* infections are presented in Tables 4 and 5, respectively.

Table 4: Body weight susceptibility of *Clarias gariepinus* to *Flavobacterium columnare* infections

No. of examined fish	Weight	No. of infected fish	%	No. of isolates
48	100-200g	-	-	-
48	201-300g	5	10.14	5
48	301-500g	2	4.16	2

Table 5: Seasonal susceptibility of *Clarias gariepinus* to *Flavobacterium columnare* infections

Seasons	No. of examined fish	No. of infected fish	%
Winter	36	-	-
Spring	36	1	2.77
Summer	36	1	2.77
Autumn	36	5	13.88

Experimental infection

Clinical signs as loss of appetite and sluggish movement began to appear on fish 48 hours post challenge. Three days later, respiratory manifestations were evident on the fish that had gill scarification. Some fish were found near the surface with open mouth gasping air, while others found at the bottom with respiratory distress. Fin rot of the dorsal and caudal fins were evident almost on all fish. Also, erosions and ulceration of the skin and pale areas were found at the base of the dorsal fin that starts small then enlarge giving the characteristic appearance of "saddle-back lesion" (Fig. 2). Out the group that had skin scarification, two fish died within 2 weeks post challenge showing large pale area and erosions at the base of dorsal fin and paleness and sloughing at the periphery of gill filaments. By the 21st day, all fish showed signs of advanced infections. Fish that had gill scarification did not show mortalities till the end of the experiment. Interestingly, fish challenged through immersion without scarification did not develop typical signs of infection described above and survived to the 21st day.

Antibiogram:

Flavobacterium columnare in the present study was highly sensitive to cefotaxim, ciprofloxacin and ofloxacin, but resistant to cephradine, and moderately to less sensitive to trimethoprim-sulfamethoxazole, E-moxclav, colistin, and flummox.

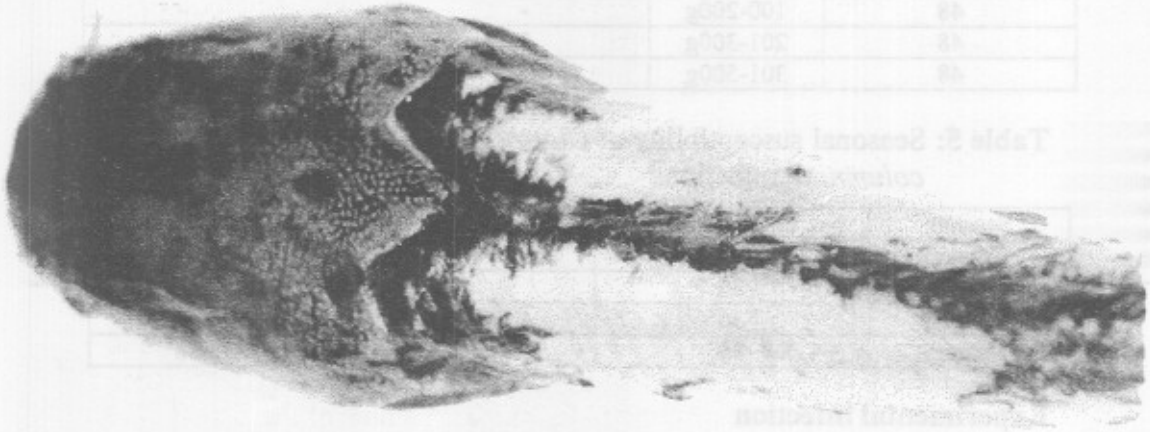

Fig. 2: Sharptooth catfish, *Clarias gariepinus*, challenged after skin scarification with $3.5X10^7$ colony forming units/ml of *Flavobacterium columnare* showing "saddle-back lesion".

DISCUSSION

Results of the present study showed that *F. columnare* causes light infections among wild sharptooth catfish in Qena, Egypt. Out of 144 fish examined over a year, 7 (4.86%) *F. columnare* strains were isolated from wild sharptooth catfish. Low prevalence of columnaris in the present study may be due to the fact that outbreaks of columnaris are rarely spontaneous and influenced by a combination of environmental and other factors that are stressful to the host (Wakabayashi, 1991) that may not occur frequently in the wild. Furthermore, Shotts and Starliper (1999) concluded that most species of fish are susceptible to columnaris following some type of environmental stress and when water temperatures are in the upper part of their preferred temperature range. Columnaris epizootics in natural populations of fish have been, however, repeatedly reported from fish in lakes, rivers and reservoirs across the US, and in anadromous salmonids in the Pacific North-West (Becker and Fujihara 1978). Also, Schachte (1983) stated that epizootics of

columnaris disease frequently occur in natural populations of fish and high losses may be observed.

Gills of sharptooth catfish naturally infected by columnaris disease were necrotic and sloughed as previously reported (Post, 1983; Inglis, *et al.* 1993 and Noga, 1996), while lesions at dorsal fin could not be detected in examined fish. Virulent strains of *F. columnaris* may attack gill tissue and cause a "gill rot" condition (Wood 1974); however, cutaneous infections seem to be more prevalent in most species of fish (Schachte, 1983).

Weight susceptibility of sharptooth catfish to *F. columnare* infections showed no specific pattern for a particular weight rendering all body weight groups examined susceptible to the diseases. This is supported by Pacha aand Ordal (1967), Schäperclaus (1992), Noga (1996), who concluded that all ages are susceptible to columnaris. Younger fish, however, are more susceptible (Schäperclaus 1992) since induction of this disease depends on the immune status of the fish.

Seasonal susceptibility of sharptooth catfish to columnaris demonstrated that the disease was more prevalent in higher temperature as was reported by Wakabayashi and Egusa, (1972), Chen *et al.* (1982) and Wakabayashi (1991). Morrison *et al.* (1981) recorded that outbreaks of saddle back disease caused by *F. columnare* occurred at 14.4-22.2°C being more severe at the higher end of the range. Columnaris outbreaks may result in high mortality during spring and autumn and are generally associated with poor environmental conditions (Shotts and Starliper 1999). Alos, Durborow *et al.* (1998) found columnaris disease to occur in channel catfish, *Ictalurus punctatus*, when temperatures are in the range of 25°C to 30°C in the spring, summer and fall. Furthermore, Decostere *et al.* (1999) mentioned that the adhesion of high virulence strain of *F. columnaris* to gills of common carp, *Cyprinus carpio* (L.), was enhanced in high temperature.

Despite its significance as a pathogen, relatively little is known about the pathogenicity of *F. columnare* and no reproducible challenge model has been developed. A widely used, but not well-documented challenge model for *F. columnare* utilizes the scarification or abrasion of the host prior to bath immersion. In contrast to the majority of fish pathogens, artificial infection by a highly virulent strain of this bacterium is more effective by contact exposure than by injection (Pacha and Ordal, 1970). Contact infection represents a more natural way of infection whereas intramuscular injection bypasses the natural defense mechanisms such as skin and mucus. In the current study, clinical signs

of the induced infection and route of induction clearly show that columnaris disease does not usually occur as a spontaneous infection but results from abrasion or scarification to fish either to gills or skin as suggested by Post (1987), Noga (1996), Durborow *et al.* (1998) and Bader *et al.* (2006). This may explain the difficulty to induce infections in the present study through immersion of non-scarified fish. In contrast, Decostere *et al.* (1999) reported that black mollies challenged via immersion in *F. columnare* bacterial suspension showed respiratory distress and behavioral changes as early as 4 hour post-immersion. Such findings could be due to difference in the virulence of strains used. Wood (1968) reported that less virulent strains of *F. columnare* caused outbreaks only at temperatures above 20° C and produced extensive tissue damage, whereas more virulent strains killed fish at temperatures as low as 15° C with no apparent gross tissue damage.

Clinical signs of sharptooth catfish experimentally infected by *F. columnare* were, mainly, skin ulcerations, fin rot that usually starts at the outer edges of dorsal and caudal fins and gills affection with sloughing of gill filaments tips. The

obtained clinical signs were nearly similar to those reported by Pacha and Ordal, (1967); Austin and Austin, (1987); Hawke and Thune, (1992); Inglis, *et al.* (1993); Noga, (1996) and Stringer-Roth, *et al.* (2002). The extensive necrosis and tissue destruction associated with *F. columnare* infections suggests that these bacteria produce strong tissue-destroying enzymes. When attached onto host surfaces, *F. columnare* release proteases to break down proteins of the host's extracellular matrix (Aumailley and Gayraud 1998 and Durborow, *et al.,* 1998), thus causing necrotic lesions (Miyoshi and Shinoda 2000).

Due to the ubiquitous presence of *F. columnare* in aquatic environments, eradication of the disease in fish farms is not likely to occur. Control and treatment of columnaris have primarily been directed towards the use of improved water-management practices to reduce physiological and environmental stress and use of surface-acting agents (Wakabayashi 1991) as *F. columnare* primarily attacks gills, skin and fins of fish. On the other hand, medicated feed are commonly used (Noga, 1996). In the present study, *F. columnare* were highly sensitive to cefotaxim, ciprofloxacin and ofloxacin, but less sensitive to colistin and flummox. These results are nearly similar to those reported by Decostere, *et al.* (1998) as they recorded that minimum inhibitory concentration (MIC) value is high for colistin. The bacteria were, also, moderately sensitive to trimethoprim-sulfamethoxazole and E-moxclav

as concluded by Decostere, *et al.* (1998) who stated that MIC values were high for sulfamethoxazole.

REFERENCES

Anacker, R.I. and Ordal, E.J. (1959): Producers for the detection and identification of certain fish pathogens. 3rd Ed. American Fish Society. Oregon, USA.

Aumailley, M. and Gayraud B. (1998): Structure and biological activity of the extracellular matrix. Journal of Molecular Medicine 76, 253–265

Austin, B. and Austin, D.A. (1987): Bacterial Fish Pathogens. Ellis Horwood, West Sussex, England, pp. 225-247.

Austin, B. and Austin, D.A. (1999): Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. Springer, New York.

Bader, J.A.; Moore, S.A. and Nusbaum, K.E. (2006): The effect of cutaneous injury on a reproducible immersion challenge model for *Flavobacterium columnare* infection in channel catfish (*Ictalurus punctatus*). Aquaculture 253(1-4): 1-9.

Becker, C.D. and Fujihara, M.P. (1978): The bacterial pathogen *Flexibacter columnaris* and its epizootiology among Columbia river fish. A review and synthesis. Monograph no. 2. American Fisheries Society, Washington, DC.

Bernardet, J.F. and Grimont, P.A.D. (1989): Deoxyribonucleic acid relatedness and phenotypic characterization of *Flexibacter columnaris* sp. noc., nom., rev., *Flexibacter psychrophilus* sp. nov., nom. Rev., and *Flexibacter maritimus* Wakabayshi, Hikida and Masumura, 1986. International Journal of Systematic Bacteriology 39: 346-354.

Bernardet, J.F.; Segers, P.; Vancanneyt, M.; Berthe, M.; Kersters, K.; and Vandamme, P. (1996): Cutting a Gordian knot: Emended classification and description of the genus Flavobacterium, emended description of the family Flavobacteriaceae, and proposal of *Flavobacterium hydatis* nom. nov. (basonym, Cytophaga aquatilis Strohl and Tait 1978). International Journal of Systematic Bacteriology 46: 128-148.

Chen, C.R.; Chung, Y.Y. and Kuo, G.H. (1982): Studies on the pathogenicity of *Flexibacter columnaris*. I. Effect of dissolved oxygen and ammonia on the pathogenicity of *Flexibacter columnaris* to eel (*Anguilla japonica*). CAPD Fisheries Series No. 8, Reports on Fish Disease research 4: 57-61.

Davis, H.S. (1922): A new bacterial disease of fresh-water fishes. Bulletin of U.S. Bureau of Fisheries 38, 261–280.

Decostere, A.; Haesebrouck, F. and Devriese, L.A. (1998): Characterization of four *Flavobacterium columnare* (*Flexibacter columnaris*) strain isolated from tropical fish. Veterinary Microbiology 62 (1): 35-45.

Decostere, A.; Haesebrouck, F.; Turnbull, J. and Charlier, G. (1999): Influence of water quality and temperature on adhesion of high and low virulence strains of *Flavobacterium columnare* to isolated gill arches. Journal of Fish Diseases 22, 1–11.

Durborow, R.; Thune, R.; Hawke, J. and Camus, A. (1998): Columnaris Disease: A bacterial infection caused by *Flavobacterium columnare*. SRAC Publication, No. 479.

Elkamel, A.A. and Thune, R.L. (2003): Invasion and Replication of *Photobacterium damselae* subspecies *piscicida* in Fish Cell Lines. Journal of Aquatic Animal Health, 15: 167-174

Ellsaesser, C.F. and Clem, L.W. (1986): Hematological and immunological changes in channel catfish by handing and transport. Journal of Fish Biology. 28: 511-521.

Finegold, S.M. and Martin, W.J. (1982): Diagnostic Microbiology. 6th Ed. C.V. Mosby, USA.

Fish, F.F. and Rucker, R.R. (1943): Columnaris as a disease of coldwater fishes. Transactions of the American Fisheries Society 73, 32–36.

Hawke, J.P. and Thune, R.I. (1992): Systemic isolation and antimicrobial susceptibility of *Cytophaga columnaris* from commercially reared channel catfish. Journal of Aquatic Animal Health 4: 109-113.

Inglis, V.; Robert, R.J. and Bromage, N.R. (1993): Bacterial diseases of fish, Blackwell Press, London.

Miyoshi, S. and Shinoda, S. (2000): Microbial metalloproteases and pathogenesis. Microbes and Infection 2, 91–98

Morrison, C.; Cornick, J.; Shum, G. and Zwicker, B. (1981): Microbiology and histopathology of saddleback disease of underyearling Atlantic salmon, *Salmo salar* (L.). Journal of Fish Diseases 4: 243-258.

Noga, E.J. (1996): Fish Diseases: Diagnosis and Treatment. Mosby, St. Louis, Missouri. 156–158.

Pacha, R.E. and Ordal, E.J. (1967): Histopathology and experimental columnaris disease in young salmon. Journal of Comparative Pathology. 77: 419-423.

Pacha, R.E. and Ordal, E.J. (1970): Myxobacterial diseases of salmonids. In: S. F. Snieszko (Eds). A symposium on diseases of fishes and shellfishes. Special Publication No. 5, American Fisheries Society, Washington, DC, PP. 243-257.

Post G. (1987): Textbook of fish Health. 2nd Ed., 182-185. T.F.H.Publication, Inc. Ltd.

Schachte, J. (1983): Columnaris Disease. In Meyer, F.P., Warren, J.W. and Carey T.G (Eds.), A guide To Integrated Fish Health Management in the Great Lake Basin. Special Publication No. 83-2. Great Lake Fisheries Commission, Ann Arbor, Michigan, USA.

Shamsudin, M.N. (1994): Pathogenesis of *Flexibacter columnaris* and immunity in the channel catfish. PhD dissertation. Auburn University, AL, USA.

Schäperclaus, W. (1992): In Schäperclaus, W., Kulow, H. and Schreckenbach K. (Eds.), Fish Diseases. 5th Ed. Vol. 1. Fischkrankheiten, Akademie-Verlag, Berlin, Germany.

Shotts, E.B. and Starliper, C.E. (1999): Flavobacterial diseases: columnaris disease, cold-water disease and bacterial gill disease. In: Woo, P.T.K., Bruno, D.W. (Eds.), Fish Disease and Disorders. Viral, Bacterial and Fungal Infections, Vol. 3. CAB Publishing, New York, pp. 559– 576

Stoskopf, M.K. (1993): Fish Medicine. Bacterial Diseases of Goldfish, Koi and Carp p.473 W. B. Saunders Co., Philadelphia USA.

Stringer-Roth, K.M.; Yunghans, W. and Caslake, L.F. (2002): Differences in Chondroitin AC lyase activity of *Flavobacterium columnare* isolates. Journal of Fish Diseases 25: 687-691.

Wakabayashi, H. (1991): Effect of environmental conditions on the infectivity of *Flexibacter columnaris* to fish. Journal of Fish Diseases. 14: 279-290.

Wakabayashi, H. and Egusa, S. (1972): Preliminary experiments on environmental factors influencing the prevalence of columnaris disease. Fish Pathology 7: 58-63.

Wood, J.W. (1968): Diseases of Pacific Salmon, their Prevention and Treatment. Washington Department of Fisheries, Hatchery Division, Olympia, p82.

Wood, J.W. (1974): Diseases of Pacific salmon: their prevention and treatment. Wash. State Dep. Fish. Olympia, WA. p82