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PATHOGENESIS OF COLUMNARIS DISEASE IN AFRICAN SHARPTOOTH CATFISH, *CLARIAS GARIEPINUS*

(With 2 Tables and 4 Figures)

By

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كيفية حدوث مرض الكولمنارس فى الأسماك القطية النيلية (القراميط الأفريقية)

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تناول هذا البحث اجراء دراسة ميدانية حول كيفية حدوث مرض اللكولمنارس فى الأسماك القطية النيلية (القراميط الأفريقية) بمحافظة أسيوط – جمهورية مصر العربية. وقد تم اكتشاف العدوى بميكروب الفلافوباكتيريم كولمنارى المسبب للمرض فى عدد 33 عينة (22,9%) من إجمالى عدد 144 سمكة تم فحصهاعلى مدار عام . هذه الدراسة أوضحت أن الإصابة بالطفيليات تزيد من عرضة أسماك القرامبط للإصابة بالعدوى ب ميكروب الفلافوباكتيريم كولمنارى وتلعب دورا هاما فى إحداث العدوى فى الطبيعة. وتضمنت هذه الدراسة إنشاء نموذج للعدوى الاصطناعية بميكروب الفلافوباكتيريم كولمنارى باستخدام الغمر مع خدش جلد أو خياشيم الأسماك. الأسماك التى تعرضت للميكروب بواسطة الغمربدون خدش لم تظهر عليها أعراض العدوى وبقيت حية حتى نهاية التجربة. تم استخدام عتره ميكروب الفلافوباكتيريم كولمنارى (SK8FC) والتى تم عزلها من جلد أسماك مصابة طبيعيا فى إجراء العدوى الاصطناعية لهذه الدراسة . وقد أدى إحداث خدش إما فى الجلد أو الخياشيم إلى تعزيز اختراق ميكروب الفلافوباكتيريم كولمنارى لجسم الأسماك المعرضة للميكروب. الأعراض الإكلينيكية والوفيات كانت أكثر عنفا وظهرت أسرع فى حالة خدش الخياشيم عنها فى حالة خدش الجلد. وكذلك تم دراسة الصبغ بواسطة الإميونوهستوكيمسترى ودراسة الهستوباثولوجى للكشف عن أعضاء الجسم التى يستهدفها الميكروب بصورة أساسية وتوزيعه والتغيرات المرضية التى يسببها. أوضح الصبغ بواسطة الإميونوهستوكيمسترى أن الجلد والخياشيم هما العضوان الأساسيان لتمركز الإصابة ب ميكروب الفلافوباكتيريم كولمنارى وهما أيضا العضوان الأساسيان اللذان يظهر عليهما التغيرات الهستوباثولوجية، كما أظهر نسيجا الجلد والخياشيم درجة صبغة عالية ل ميكروب الفلافوباكتيريم كولمنارى فى المجموعات التى تم خدشها عنه فى المجموعة التى لم يتم خدشها.

SUMMARY

This study was conducted to investigate the pathogenesis of columnaris disease in African sharptooth catfish, *Clarias gariepinus*. *Flavobacterium columnare* infections were detected in 33 (22.9%) fish out of 144 fish collected and examined over a year, in Assiut, Egypt. The present study

demonstrated that parasitic infestation increases the susceptibility of fish to columnaris disease and plays an important role in initiation of natural infection. A reproducible model of experimental infection was developed to help studying the pathogenesis using immersion with either skin or gill scarification of challenged fish. Fish challenged through immersion with scarification developed severe signs of infections and showed mortalities, while fish challenged without scarification did not develop typical signs of infection and survived until the end of the experiment. *F. columnare* strain SK8FC isolated from skin of naturally infected fish was used throughout the challenge study. The invasion of *F. columnare* was enhanced by either skin or gill abrasion. Clinical signs and mortalities were more severe and rapidly developed in the gill-scarified group than in skin sacrificed group. Immunohistochemistry staining and histopathology studies were conducted to investigate the target organs, the distribution of the bacterium, and the pathological changes. Immunohistochemical staining demonstrated that the skin and gills were the main organs of *F. columnare* localization and the main organs expressing histopathological alterations. Skin and gill tissues were more strongly stained for *F. columnare* in scarified groups than in non-scarified group.

Key words: *African sharptooth catfish, Flavobacterium columnare, pathogenesis, immunohistochemistry, challenge model.*

INTRODUCTION

Flavobacterium columnare has been recognized as a worldwide pathogen of freshwater fish that causes columnaris disease, one of the oldest known fish diseases in the world. It affects a wide variety of fish including wild, cultured and ornamental fish in both freshwater and saltwater environments (Shamsudin 1994; Plumb 1999 and Shotts and Starliper 1999). The ubiquitous distribution of *F. columnare* in fresh water environments and the tendency of fish to acquire the disease after mechanical and/or environmental stress make *F. columnare* among the most detected pathogens in cultured, ornamental, and wild fish populations (Shamsudin and Plumb 1996; Shotts and Starliper 1999).

Columnaris disease leads to significant losses and high mortalities, especially during spring and autumn (Pacha and Ordal 1970 and Becker and Fujihara 1978), and more to be expected when fish are stressed (Durborow *et al.*, 1998). Clinical signs are restricted to the external surface of fish, mainly skin and gills. Skin ulcerations started at the base of the

dorsal fin and then extended laterally around the body. In addition, fin rot and respiratory manifestations are common.

Despite the increasing significance of the disease, the actual mechanism of pathogenesis of *F. columnare* infections has only been partially elucidated, hampering the development of preventive measures to efficiently combat this disease condition (Nematollahi *et al.*, 2003). Little is known about *F. columnare* infections in African sharptooth catfish, *Clarias gariepinus*, therefore, unlocking the pathogenesis and virulence mechanisms of *F. columnare* may ultimately provide the knowledge necessary for designing more effective means of control.

Because of the difficulties in reproducing the *F. columnare* infections experimentally, previous studies describing the infections in African sharptooth catfish usually relied on natural infections. There are no standardized models of experimental infection, enzymatic, immunological, or genetic methods that can be used economically to study *F. columnare* in clinical situations and this has further deterred understanding the pathogenesis of the infections. In addition, most previous studies focused on describing the clinical signs and lesions (Ahmed *et al.*, 2007), rather than investigating the porta of entry, invasion, site of localization, factors that help induction of the disease.

The present study was conducted to investigate the pathogenesis of columnaris in African sharptooth catfish through developing a model of experimental infection, studying how the infections start, detecting the porta of entry and the target organs, and developing an immunohistochemistry technique to detect the site where the bacteria localize during the infection.

MATERIALS and METHODS

Fish collection:

A total number of 144 alive African sharptooth catfish, *Clarias gariepinus* were randomly caught from different localities of The River Nile and El-Ibrahemia canal, Assiut City, over a year (12 fish/month). Collected fish have a body weight range of 120 to 900g and total length range of 25 to 55cm. Fish were transported immediately to the Aquatic Animals Wet Lab., Veterinary Hospital Clinic, Faculty of Veterinary Medicine, Assiut University to be examined.

Clinical and Bacteriological Examination of fish Samples:

Fish were examined for clinical signs, external and possible internal lesions according to Stoskopf (1993). Opercula were removed to expose the gill tissues and samples were cultured onto cytophaga agar medium

(Anacker and Ordal, 1959) and incubated at 25º for 48 hours. Samples from the skin mucus, fins, liver, spleen and kidney were also cultured directly on cytophaga agar medium.

Identification of bacterial isolates:

Purified bacterial isolates were identified based on the colony morphology, color and cultural characteristics on cytophaga agar, microscopic examination (Gram stain and motility test), various biochemical characters that include flexirubin pigment test, oxidase, catalase, indole, voges proskauer, methyl red, H_2S production and carbohydrate utilization (lactose, glucose, arabinose, raffinose, xylose, sucrose and maltose) according to Cruickshank *et al.* (1975) and Austin and Austin (1987).

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

Fish: A total number of 254 apparently healthy *C. gariepinus* with an average body weight of 80-120 g and total length range of 20-27 cm were obtained from fish ponds at El-Minia Governorate and used for experimental challenge. Fish were housed in 400 L tanks at the Aquatic Animal Wet Lab., and 20 fish were randomly examined to exclude *F. columnare* infections. Water was circulated at an exchange rate of 0.5 L/min, and daily dissolved oxygen was 6.5±0.2 mg/L and temperature was maintained at 25±1ºC. Fish were acclimated for 2 weeks in 100 L glass aquaria according to the protocol of maintaining bioassay fish described by Ellsaesser and Clem (1986).

Rapid haemagglutination test: This test was performed to detect the presence of a haemagglutinating agent in the pathogen according to Grimes (2002). Briefly, a drop of 10% catfish red blood cells was placed on a clear glass slide. One drop of Phosphate buffered saline (control) or bacterial suspension of selected *F. columnare* strains was added. Positive agglutination was indicated by clumping in red blood cells. Based on the intensity and time of onset of clumping, *F. columnare* strain (SK8FC) was selected to conduct the experimental challenge study. There is a virulence relationship between HA activity and *F. columnare* infectivity in channel catfish and it was noted that a positive relation existed between *in vitro* epithelial cell adherence and isolate virulence (DelCorral, 1988).

Bacterial strain: *F. columnare* strain (SK8FC) isolated from skin of infected fish was passed three times through skin scarification of African sharptooth catfish to be used for experimental challenge.

Bacterial challenge suspension and counts: Colony forming units (cfu) counts in bacterial suspensions were determined using spectrophometery optical density values at wavelength of 600nm and standard-plate-count method with ten-fold serial dilution (Elkamel and Thune 2003). Counts

were determined on BHI plates with 4% agar to inhibit gliding activities of *F. columnare*. The challenge suspension was prepared by growing *F. columnare* in BHI broth at 25°C until mid-log phase (optical density of 0.5 that equals to 5.6×10^6 cfu/ ml), then the dilution was made to a final concentration of 1.4×10^6 cfu/ml.

Experimental infection: Acclimated *C. gariepinus* were divided equally into 6 groups (13 fish each). Fish of each group were challenged through immersion for 20 min in the challenge suspension. *F. columnare* bacterial suspension was used to challenge 3 groups. The first group was challenged with scarification of skin at the base of dorsal fin, while the second group was challenged with scarification of gills (Fish and Rucker 1943). The third group was challenged without any scarification. Two control groups were similarly treated as challenged groups by either skin or gill scarification, but immersed in the same volume of sterile BHI broth with no bacteria, while a third control group was immersed in the same volume of sterile water with no scarification (Table 1). Clinical signs and mortalities were recorded daily over 15 days and re-isolation and identification of bacteria was done from freshly dead and moribund fish as described above. The whole experiment was repeated three times.

Table 1: Experimental infection of African sharptooth catfish, *Clarias gariepinus*, with *Flavobacterium columnare*.

Group	Immersion suspension	Route
Challenged	*Flavobacterium columnare* (1.4×10^6 cfu/ml)	Skin scarification
		Gill scarification
		No scarification
Control	Sterile BHI broth	Skin scarification
	Sterile BHI broth	Gill scarification
	Sterile water	No scarification

Histopathological examinations:

Small tissue specimens of moribund challenged fish were collected from the skin and gills and rapidly fixed in 10% neutral buffer formalin (Bullock, 1989). Fixed specimens were processed and paraffin sections were prepared and stained with hematoxylin and eosin for microscopical examination according to Drury and Wallington (1980).

Immunohistochemistry:

Preparation of rabbit antisera against *F. columnare*: Antisera against *F. columnare* were prepared according to Anderson and Dixon (1984). Briefly, two-male New Zealand White rabbits (*Oryctolagus cuniculus*),

each weighing about 2 kg were injected subcutaneously and intramuscularly with 5 ml of *F. columnare* bacterin that was previously prepared according to Garvey *et al.* (1977) and 1 ml Freund's complete adjuvant (Sigma-Aldrich, St. Louis, MO, USA). Three weeks post initial inoculation, the rabbits were re-injected with 5 ml of *F. columnare* bacterin without adjuvant.

One week post the 2nd inoculation, inoculated rabbits were anaesthetized and blood was collected from the eyes. Serum was collected by centrifugation, and stored at -20 ºC. To estimate the antibody titer, the pooled sera against *F. columnare* were analyzed by tube agglutination method. The best working serum dilution was found to be 1:40 of the original pooled sera.

Staining: Tissue samples of the skin and gills of experimentally infected *C. gariepinus* were fixed in 10% buffered formalin and embedded in paraffin for immunohistochemical processing. Staining was performed using immunohistochmistry Kit (Ultravision Detection System, Suffolk, UK) according to the manufacturer's instructions.

RESULTS

Clinical Examination:

Examined wild fish showed a wide variety of skin and gill lesions. The main clinical signs observed on naturally infected fish were erosions and ulceration of skin at the base of the dorsal fin and on the head (Fig. 1), and fin rot with separated fin rays, in addition to paleness of gill color and sloughing of gill filaments. Microscopic examination of wet mounts from eroded areas of infected skin or gills revealed the presence of masses of long bacterial cells.

Parasitological examination:

Parasitological examination of the 144 fish collected in the present study revealed that 114 (81.4%) fish were infested with *Quadriacanthus sp.*, *Trichodina sp.*, *Icththyophthirius sp.*, *Henneguya sp.* or digenea trematodes, while only 30 fish were clinically negative for external parasites.

Bacteriological isolation and identification:

Bacteriological examination of collected samples resulted in recovery of 112 suspected *Flavobacterium* isolates from skin, gills and kidney of the 144 examined fish. Primary identification of isolates was done according to cultural and morphological characters, while comprehensive identification of these isolates was done according to their biochemical characters (Austin and Austin 1987) and 36 isolates was identified as *Flavobacterium columnare* (Table 2).

Table 2: Phenotypic characteristics of suspected *Flavobacterium columnare* isolates.

Biochemical reactions	*Flavobacterium columnare*
Gram stain	Gram negative
Flexirubin pigment	35/36
Gliding motility	36/36
Oxidase	36/36
Catalase	31/36
Indole	3/36
Vogus Proskauer	0/36
Methyl red	0/36
H_2S production	28/36
Glucose	1/36
Sucrose	0/36
Raffinose	3/36
Maltose	2/36
Lactose	0/36
Arabinose	0/36
Xylose	1/36

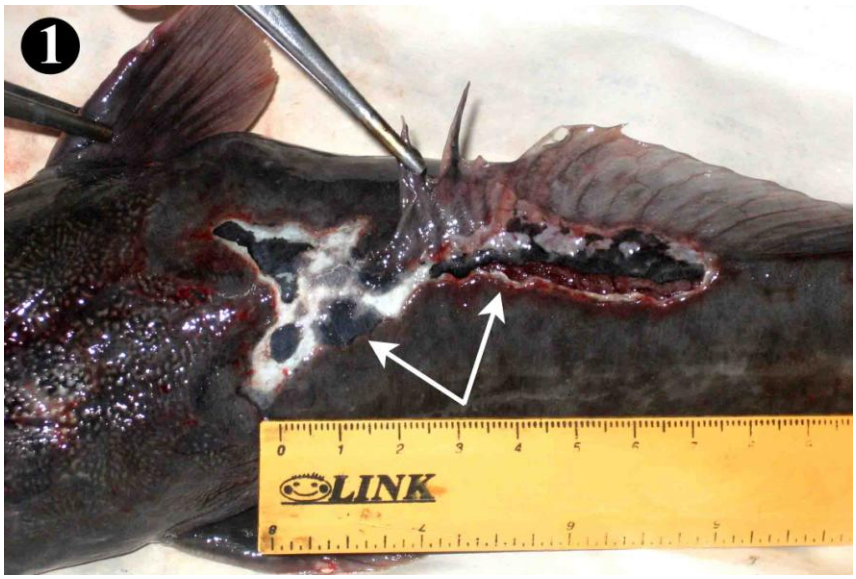

Fig. 1: African sharptooth catfish, *Clarias gariepinus*, naturally infected with *Flavobacterium columnare* showing ulcer (arrow) in the head area and at the base of the dorsal fin.

Prevalence of natural infection:

Thirty six *Flavobavterium columnare* strains were isolated from 33 (22.9%) out of 144 African sharptooth catfish examined. *F. columnare* was isolated from gills, skin and kidney of the naturally infected fish, while was not isolated from either liver or spleen. Results indicated that *F. columnare* was isolated from the gills (51.52%) and skin (48.48%) at significantly higher rates ($p<0.05$) than from kidneys (9.09%). Out of the 33 infected fish, mixed infection of *F. columnare* and parasitic infestation) were detected at significantly higher rates (75.76%), where $p<0.05$, than in fish with only flavobacterial infection (24.24%).

Experimental infection

1- Fish infected through immersion with skin scarification:

Clinical signs and mortalities began to appear within 24 hours post challenge. Loss of appetite, sluggish movement, loss of balance, frayed fins and fin rot especially in dorsal fin were evident. By the end of the first day, the average number of dead fish was 3, and by the second day 3.33 fish died. The survived fish after the third day showed respiratory manifestations and fish were found near the water surface opening their mouth and gasping air, while others were moribund near the bottom with respiratory distress. By the fifth day, 3.67 fish were dead showing ulceration and loss of skin on both sides of the body (Fig. 2), started small at the base of the dorsal fin then enlarged to encircle the entire body giving the characteristic appearance of "saddle-back lesion" and pale gills at necropsy. After 15 days, fin rot of the dorsal and caudal fins were evident on all fish survived. The average mortality rate in this group was 76.9%.

2- Fish infected through immersion with gill scarification:

Challenged fish showed the same described signs as those appeared on fish challenged with skin scarification but with faster onset and more severity. Shortly, post challenge rapid onset of skin loss, loss of balance and vertical hanging of fish were evident. After only about 8 hours, fish started to die with almost complete loss of skin. Marked respiratory manifestations were clearly observed 12 hours post challenge. Then, 3.67 fish died 18 hours post challenge. Two days later, all the remaining fish were dead with erosions, ulceration and loss of the skin. At necropsy, pale coloration of gills with sloughing at the periphery of gill filaments were observed. The average mortality rate in this group was 100%.

3- Fish infected through immersion without scarification:

Interestingly, these fish survived till the end of the experiment and did not develop typical signs of infection. Only one fish in two replicates of the experiment showed loss of skin and died by the third day post challenge. The average mortality rate in this group was 7.7%.

4- The control groups:

The control fish did not show any clinical signs and remain alive without mortalities after the 15th day of the experiment.

Fig. 2: African sharptooth catfish, *Clarias gariepinus*, challenged by $1.4X10^6$ colony forming units/ml of *Flavobacterium columnare* with skin scarification showing complete loss of epidermis (arrow points to the site of skin scarification).

Histopathology:

The skin and gills of African sharptooth catfish were the main organs affected. In the present study, fish exposed to *F. columnare* without scarification had intact epidermis. Myofibrillar degeneration, muscular hemorrhage and few inflammatory cellular reaction between the muscle bundles were, however, observed when compared to the control. While, the skin of fish exposed to *F. columnare* with either skin or gill scarification had complete absence of the epidermis layer (Fig. 3B).

Sever hyperplastic changes were observed in the gills of fish exposed to the bacteria and skin scarification (Fig. 3C). The gills of challenged catfish without scarification had slight interlamellar hyperplasia. While, the gills of fish being scarified and exposed to the bacteria showed necrosis of the interlamellar epithelium as well as the epithelium of the secondary gill lamellae in comparison to the control gills (Fig. 3D).

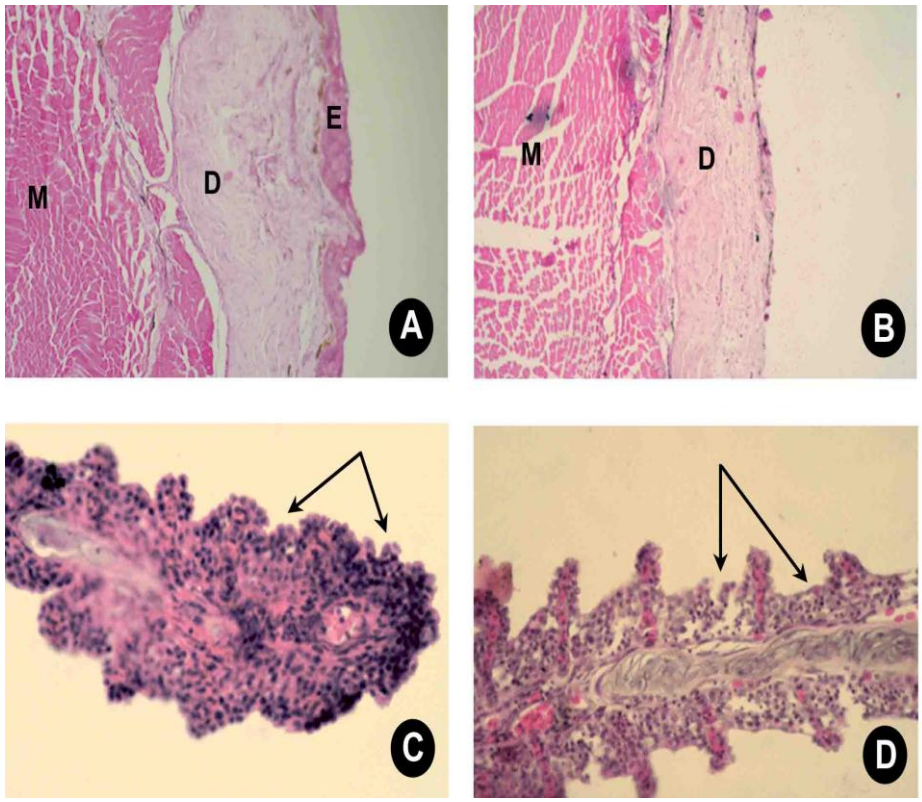

Fig. 3: (A) Normal skin of African sharptooth catfish, *Clarias gariepinus*, showing the epidermis (E), dermis (D) and muscular layer (M). (B) Skin of *C. gariepinus* exposed to *Flavobacterium columnare* with skin scarification showing complete absence of epidermis. (C) Gills of *C. gariepinus* exposed to *F. columnare* with skin scarification showing sever hyperplastic changes of the interlamellar epithelium (arrows). (D) Gills of *C. gariepinus* exposed to *F. columnare* with gill scarification showing necrosis of the interlamellar epithelium (arrow) and the epithelium of the secondary lamellae. Hematoxylne and Eosin. X=128

Immunohistochemistry:

In the present investigation, flavobacterial antigens were detected by immunohistochemistry in skin and gill sections of *C. gariepinus* challenged with *F. columnare*. Strong positively stained bacterial antigen were often found in all layers of skin and gills in catfish which challenged by bacteria and scarification either in skin or gills. The bacterial antigen

was localized by a polyclonal antibody raised in rabbit and subsequent detection steps resulted in a brown-colored product that can be visualized by light microscopy (Fig. 4).

The immunostaining of tissue sections from fish challenged with *F. columnare* without scarifications showed weak positive-stained flavobacterial antigen in all layers of skin and gills except the primary lamellae of gills. No immunostaining was observed in skin and gills of control groups (Fig. 4).

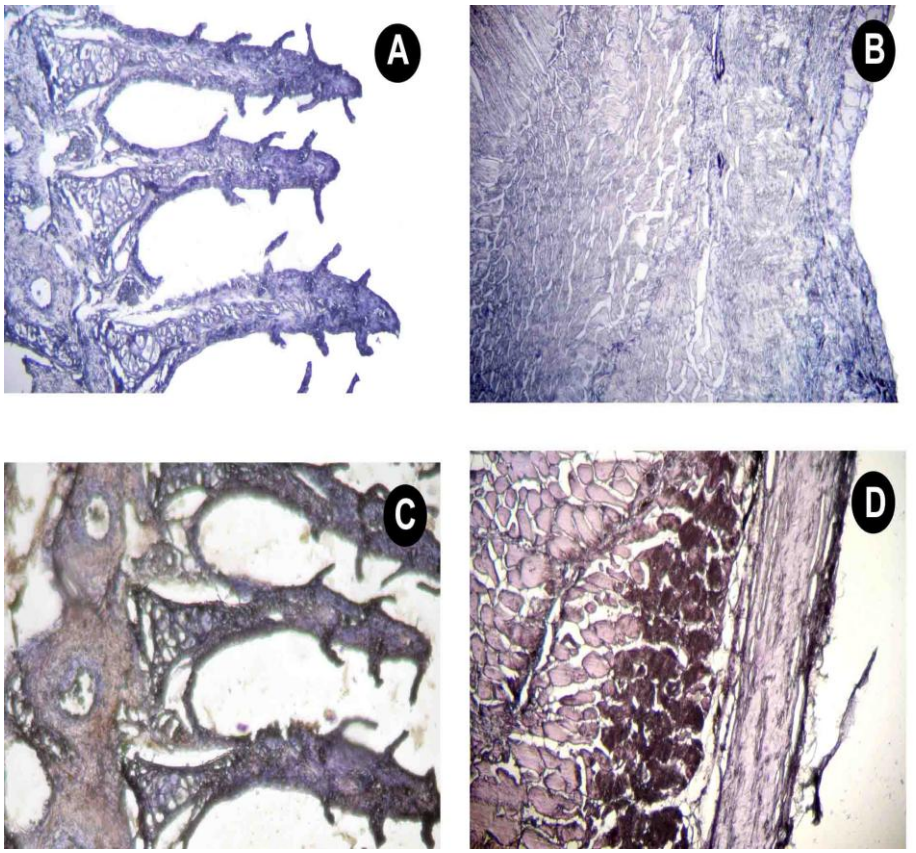

Fig. 4: Immunolocalization of flavobacteria in skin and gills of *Clarias gariepinus*. (A) Gills of the control group. (B) Skin of the control group. (C) Gills of *C. gariepinus* exposed to *Flavobacterium columnare* challenge with gill scarification showing brown pigmentation of the flavobacterial antigen. (D) Skin of *C. gariepinus* exposed to *F. columnare* challenge with skin scarification showing brown pigmentation of the flavobacterial antigen

DISCUSSION

The present study was conducted to study the pathogenesis of columnaris in African sharptooth catfish. This study investigated how the infections started and factors affecting their induction, the target organs, and the clinical signs resulted from advancing of the infections. Moreover, a model for experimental challenge of African sharptooth catfish with *F. columnare* was developed, in the present study, that helps to further study the pathogenesis of the infections.

The present study proved that *F. columnare* causes considerable infections among wild African sharptooth catfish in Assiut, Egypt. *F. columnare*, the causative agent of columnaris disease (Austin and Austin 1987), has been identified as one of the most problematic pathogens in the freshwater finnish farming industry (Suomalainen *et al.*, 2005). Despite its significance as a pathogen, relatively little is known about the pathogenicity of *F. columnare*, and virulence mechanisms by which it establishes the infections (Kunttu *et al.*, 2009). A key point in the definitive control of flavobacterial infections is to understand its pathogenesis and what make them successful pathogens. The mechanism(s) by which flavobacteria establishes an infection remains largely unexplored and the literature is somewhat contradictory.

In the present study, a reproducible model for the production of experimental *F. columnare* infections in African sharptooth catfish was developed to better understand the pathogenesis of the disease in catfish. A widely used, but not well-documented challenge model for *F. columnare* utilizes the scarification or abrasion of the host prior to bath immersion (Bader *et al.*, 2003).

In contrast to the majority of fish pathogens, artificial infection by a highly virulent strain of *F. columnare* is more effective by contact exposure than by injection (Pacha and Ordal, 1970). Contact exposure also represents a more natural way to produce columnaris infection than injection whereas intramuscular injection bypasses all the natural defense mechanisms such as skin and mucus. In experimental models with *F. columnare*, clinical signs of the induced infection do not usually occur as a spontaneous infection but requires some type of physical or environmental stress like abrasion or scarification to gills or skin as suggested by Post (1987), Noga (1996), Durborow *et al.* (1998) and Bader *et al.* (2003).

The experimental challenge in the current study proved that skin or gill "injury" is essential to establish *F. columnare* in African sharptooth catfish as was demonstrated by the groups that were challenged only with

the bacteria but without skin or gill scarification. Such groups failed to develop the typical *F. columnare* infections and pathology. On the other hand, groups that were challenged with the bacteria and with either gill or skin scarifications developed severe clinical signs and lesions associated with *F. columnare* infections. Interestingly, fish challenged without scarifications did not show massive invasion and localization of the bacteria in the gills or skin as was demonstrated by the immunohistochemistry staining study. Cutaneous injury is used for that model because a relationship between handling abrasion and columnaris disease has been demonstrated for catfish (Hussain and Summerfelt 1991; Hawke and Thune 1992). Physical injuries had been historically used to mimic actual handling and netting injuries (Davis 1922 and Fish and Rucker 1943).

The relationship between external body injury and establishing *F. columnare* infection was further, supported by the finding that mixed infections with *F. columnare* and parasites were detected at significantly higher rates (75.76%) than in parasite-free fish (24.24%). These findings clearly prove that skin abrasion or gill damage act as the main porta of entry to fish and is essential to induce the infection with *F. columnare* in African sharptooth catfish.

Rintamäki Kinnunen *et al.* (1997) reported that 30% of fish with a parasitic infestation had a simultaneous flavobacterial infection. Parasitic infestations can subject fish to microbial infections in two ways. Ectoparasites may enhance the invasion of microbes by disrupting skin mucus. Furthermore, penetrating endoparasites or blood sucking parasites may act as vectors carrying pathogens directly into the tissues of the host (Cusack and Cone 1986 and Ahne 1985). Parasites enhanced bacterial invasion in fish has been reported also in fish farming conditions (Cusack and Cone 1986; Rintamäki-Kinnunen and Valtonen 1997 and Ravichandran *et al.*, 2001). *Argulus coregoni* was reported to increase the susceptibility of rainbow trout to columnaris disease through the damage caused to fish epidermis that might open a direct entrance site of *F. columnare* (Bandilla *et al.*, 2006).

Immunohistochemistry results of the present study clearly indicated that the main target organs for invasion and/or localization of *F. columnare* in African sharptooth catfish are the gills and skin. Results showed that *F. columnare* is present in the interlamellar tissue of primary and secondary lamellae of gills and in the dermis and muscular layer of the skin of fish challenged with either gill or skin scarification.

Target organs findings were further supported by the results of direct isolation of the bacteria from naturally infected cases, where

F. columnare was isolated from the gills (51.52%) and skin (48.48%) at significantly higher rates than from kidneys (9.09%), and was not isolated at all from the liver and spleen. In addition, clinical signs of challenged were limited to either skin or gills. Clinical signs and mortalities were, however, more severe and rapidly developed in the gill-scarified groups than in the skin-scarified groups. Such findings may be attributed to more stress generated by gill scarification than by skin scarification.

Recent research has demonstrated that *F. columnare* exhibits a chemotactic response toward mucus of the skin of catfish (LaFrentz and Klesius 2009). Additionally, Staroscik and Nelson (2008) suggest that *F. columnare* was able to utilize skin mucus from Atlantic salmon, Salmo salar, as a growth substrate. These findings may, also, explain why the bacteria were isolated at such significantly higher rates from skin than from other internal organs African sharptooth catfish in the present study.

Immunohistochemistry results of the current study proved that the establishment of *F. columnare* infection in African sharptooth catfish relies on its ability to adhere onto gill or skin tissues as was previously reported with other species of fish (Decostere *et al.*, 1998 and Decostere *et al.*, 1999). It was suggested that the pathogenicity of *F. columnar*e can be divided into two phases. In the first phase, the affinity and capability to adhere onto fish play a major role in the virulence of the strain. In the second phase, adhered bacterial cells start to divide and produce lyases and proteases, which degrade fish connective tissue establishing visible signs of the disease (Suomalainen, 2005).

Clinical signs observed on African sharptooth catfish either naturally infected or challenged with *F. columnare* were nearly similar to those previously reported (Post, 1983; Plumb, 1994; Noga, 1996 and Durborow *et al.*, 1998). The commonest symptom is erosion and necrosis of the skin tissue around the dorsal fin, which sometimes even reach the spine and extensive necrosis of the gill tissue (Tripathi *et al.*, 2005). Fins affected usually have necrotic lesions on the outer edges then progress down the fin rays (Post, 1983). Severe osmotic stress that resulted from acute skin loss might be the main cause of mortalities of African sharptooth catfish in the present study. Extensive damage of the skin causes severe loss of osmotic regulation and electrolyte imbalance, which eventually causes death to the fish as was reported by Tripathi *et al.* (2005). One common clinical sign of the disease is the pronounced erosion and necrosis of the gills which is often a major site of damage (Davis, 1922). Generally, all signs observed reflect the lesions found microscopically in the histopathological studies of the present study.

There were extensive necrosis and tissue destruction associated with *F. columnare* infections in the present study as was demonstrated in the clinical signs as necrosis of gills lamellae and total destruction of the epidermal layer and in histopathological studies as necrosis of the lamellar and interlamellar epithelia. These findings suggest that this bacterium produces strong tissue-destroying enzymes that degrade proteins and connective tissue, and potentially contribute to its ability to cause disease. One such enzyme, chondroitin AC lyase, is capable of degrading acidic polysaccharides of the extracellular matrix that play a role in several processes including cellular adhesion (Jackson *et al.*, 1991). The production of extracellular proteases is an important virulence mechanism of the bacterium (Song *et al.*, 1988; Bernardet 1989; Griffin 1991; Bertolini and Rohovec 1992; Teska 1993 and Plumb 1999). These proteases have been found to contribute to direct tissue damage and/or invasiveness.

The present study proved that *F. columnare* produces infections and pathology in African sharptooth catfish. By developing an experimentally challenge model and an immunohistochemical approache, the port of the bacterium entry, how the infections do start, and where the bacteria do localize were investigated and determined. The present study is a corner stone in fully understanding the pathogenesis of *F. columnare* in African sharptooth catfish; however, further extensive studies are necessary.

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