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Columanaris infection among cultured Nile Tilapia *(Oreochromis niloticus)*

Columanaris Infektion bei gezüchteten Nil Tilapias
(Oreochromis niloticus)

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1 Introduction

Pathological gill changes have a detrimental effect on the health of fish and threaten their survival. Bacteria belonging to the genus *Flexibacter* have been incriminated by various authors as the main cause of gill affections among cultured fish all over the world (POPP, 1980; RICHARDS et al., 1985). SNIESZKO (1981) differentiated between columnaris disease caused by *Flexibacter columnaris* and bacterial gill disease caused by other felxibacteria. The former is characterized by gill necrosis while proliferation of gill epithelium is usually associated with the later.

The reports of AVAULT et al. (1968); BALARIN and HATTON (1979) and ROBERTS and SOMMERVILLE (1982) on the presence of filamentous bacteria in cultured tilapia suffering from gill affections urged us to trace out of the role of *Flexibacteria* spp. in causing losses among the most popular fish *Oreochromis niloticus* in Egypt.

2 Material and Methods

2.1 Fish

Both gills of 416 one year old cultured *O. niloticus* (El-Serow fish farm, Menia Governate) were examined throughout the course of this study. This farm was with a history of repeated mortalities associated with respiratory disorders.

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2.2 Bacterial examination

A total of 165 gill smears from diseased fish were streaked on Cytophaga agar medium (ANACKER and ORDAL, 1959) and were incubated at 28° C for 3 days. The suspected colonies (swarming, rhizoid) were picked and inoculated on different media (Nutrient, 5% sheep blood and MacConkey agar) and were incubated at the same temperature mentioned above.

Colonial, morphological and biochemical character of the isolates were studies according to the methods described by CRUICKSHANK et al. (1975), while the procedures and interpretation of the antibiotic sensitivity test by that of TREAGEN and PULLIAM (1982) (Tab. 2).

2.3 Experimental infection

This was carried out on groups (3 animal each) of *O. niloticus* fingerlings (12 cm + 1) with seven identified *F. columnaris isolates.*

The way of infection were the following (Tab. 3):

Group (A):
Bathing in water containing 18 hour old *F. columnaris* Culture (2 x 10^3 living bacterial cells/ml water).

Group (B):
Installation of 1 ml 18 hours old emulsified bacterial colonies (2 x 10^4 living bacterial cell/ml) on scarified gills. The gills were injured during smearing with a sterile glass coverslide.

Group (C):
Intramuscular injection with 1 ml of 18 hours old (2.16 x 10^4 living bacterial cells/ml) culture of isolates 3 and 7.

Group (D):
Same as group B but the fish were kept after installation in water containing 168 mg NH_4Cl/l so as to resemble the effect of accumulation of organic matter according to the method described by WALTERS and PLUMB (1980) and modified by SOLIMAN (1984).

All fish were kept following infection in 200 l glass aquaria supplied with aereated autoclaved water for an observation period of 2 weeks.

2.4 Histpathological technique

Gills of naturally and experimently infected fish were paraffinized, sectioned (5-7u), stained with Haematoxylin and Eosin (H and E) and examined microscopically for histopathological altrations.

3 Results

3.1 Natural infection

Out of 416 fish examined, 165 (39.66 percent) animals were suffering from respiratory disorders. The highest incidience was recorded during summer (47 percent) and autumn (43.2 percent) during which 13 isolates of *F. columnaris* were recovered from diseased fish (Tab. 1). The diseased fish swam near the water surface at its inlet with rapid opercular movements. The gill filaments were covered with a thick film of turbid mucous.

Tab. 1: Incidence of F. columnaris among cultured *O. niloticus*

Season	Average water temperature	Number of exemined fish	Number of clinically diseased fish	% of fish from which F. columnaris was isolated	no. of isolates
Winter	15°C±2	100	30 (30%)	0	0
Spring	20°C±2	105	40 (38.1%)	0	0
Summer	26°C±2	100	47 (47%)	12.7	6
Autumn	23°C±2	111	48 (43.2%)	14.5	7
Total		416	165 (39.66%)		13

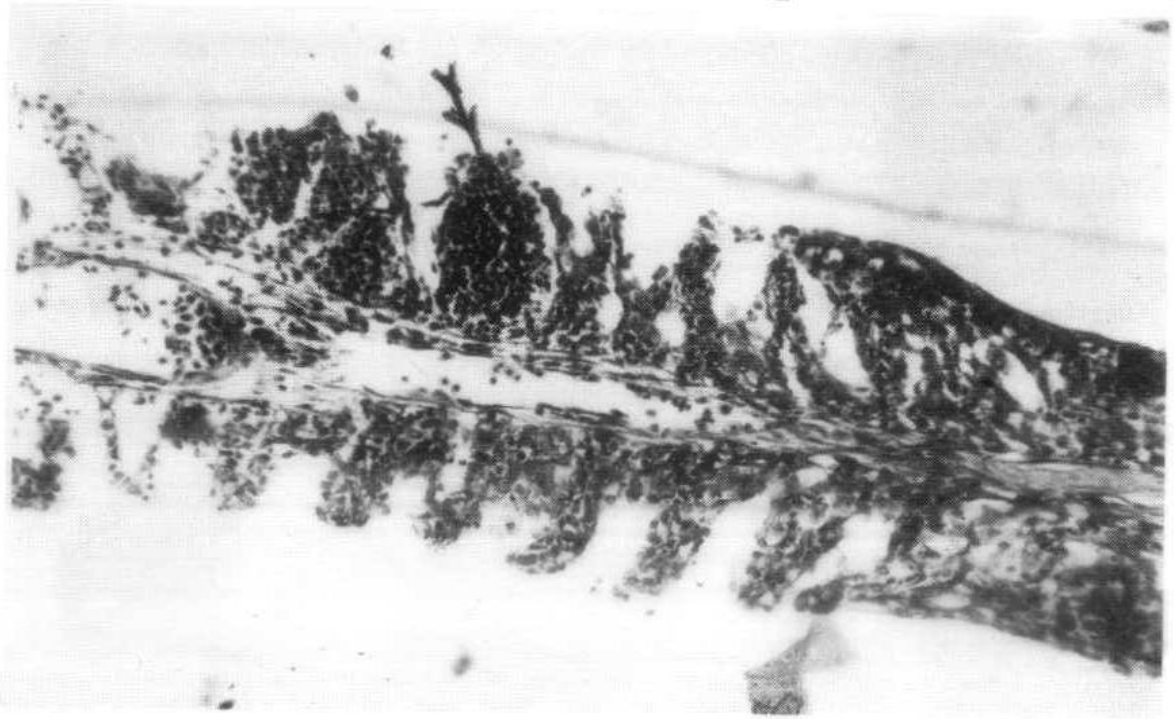

Fig. 1: Gill lamella notice: dilation of lamellar vessels, telang-ectasia (arrow), hyperplastic proliferation of lamellar epithelium and fusion of secondary lamellae H and E stain (x 200)

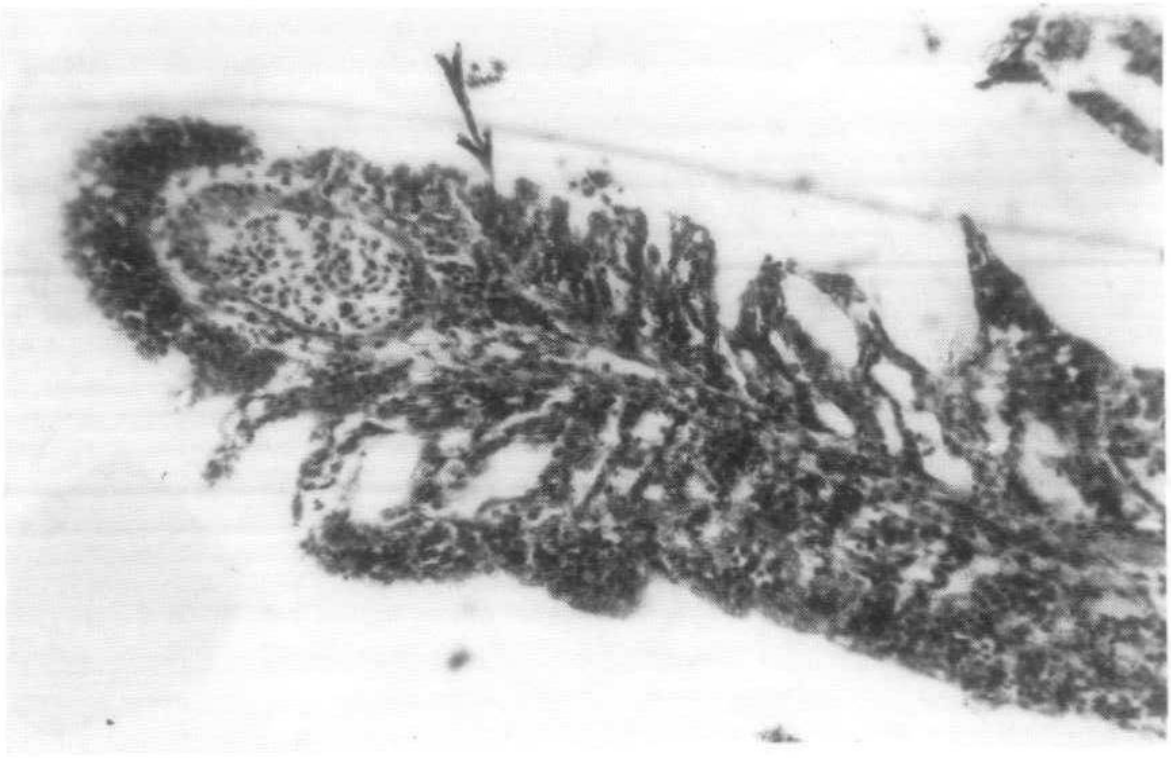

Fig. 2: Gill lamella, notice: proliferation and fusion of gill lamellae toward the tip of gill filament and necrosis on opposite side of filaments (arrow). H and E stain (x 200).

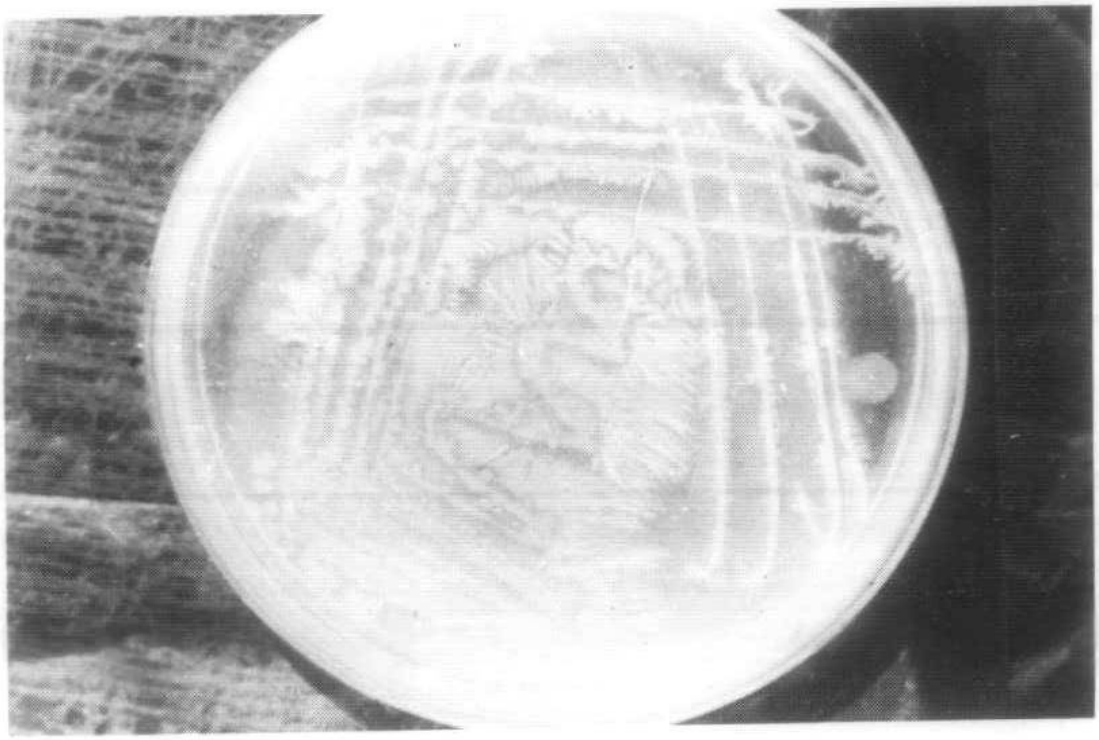

Fig. 3: *F. columnaris on cytophaga agar medium, notice: bacterial growth, showed swarming and rhizoid shape.*

Histological examination of stained gill sections of infected fish revealed dilatation of the blood vessels in gill arches, primary gill filaments and capillary beds within the secondry gill lamellae (Telangectasis) in all examined cases. Moreover, focal areas of hyperplastic proliferation of the lamellar epithelium with consequent fusion of the adjacent secondary gill lamellae (Plaque-like) were observed in many areas particularly at the base of the gill filaments (Fig. 1). The proliferative changes extended in most of the cases towards the tips of the gill filaments in fusion of secondary gill lamellae. As a results, the gill filaments appeared as masses of proliferated epithelium. On the other hand, degenerative and necrotic changes in the form of cytoplasmic vaculation, pyknosis and karyolsis were observed in the outermost cells of the proliferated epithelial masses (Fig. 2).

3.2 Bacteriological identification

The 13 isolates showed unique morphological, cultural and biochemical characters.

Cytophaga agar medium streaked with affected gill tissue and incubated at 28°C for 24 hours revealed flat, rapidly growing yellow or golden colonies with irregular stellate edges. 24 hour later, the bacterial growth showed swarming and rhizoid shape and was difficult to remove from the medium (Fig. 3). On sheep blood agar, colonies appeared flat, smooth, moist 1-2 mm in diameter with a 3-5 mm clear zone of B- haemolysis. Neither swarming nor pigment production were observed in the bacterial growth on blood or nutrient agar medium. No growth appeared on McConkey or S-S agar medium. The organism grew well at 22° C, however, slight growth was observed below 10°C and no growth at 37°C.

The highly motile, gram negative, flexible, long and thin rod (4-8 μ in length x 0.5-0.7 μ in width) bacteria showed the following biochemical characters:

- Fermentation of lactose, sucrose, glucose, maltose, and galactose .. (-)ve
- Indole, methyl red, V-P test ..... (-ve)
- Galactose ..... (+)ve
- H_2S production ..... weak
- Utilization of citrate ..... (-)ve
- Liquification of gelatin ..... (+)ve
- Nitrate reduction ..... (-)ve
- Urea hydrolysis ..... (-)ve

The antibiotic sensitivity of obtained isolated revealed that the seven tested isolates reacted in somewhat different manner to the used antibiotic discs. Thus, almost all strains were highly sensitive to Oxytetracycline, chloramphenicol and erythromycin while were moderately sensitive to streptomycin (with the exception of isolate 3 and 7). On the other hand, they were all resistant to bacitracin. The isolates were also less sen-

sitive to Colistin sulphate, Mandelamine, Novobiocin, Vancomycon and Ampicillin- (Tab. 3).

Tab. 2: Antibiotic sensitivity test of F. columnaris isolates

Antibiotics	*Isolate Number*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Streptomycin (10 mg)	++	+	+++	+	++	+	+++
Oxytetracycline (30 mg)	+++	+++	+++	+++	+++	+++	+++
Chloramphenicol (10 mg)	+++	+++	+++	+++	+++	+++	+++
Erythromycin (13 mg)	+++	+++	+++	+++	+++	+++	+++
Colistin sulphate (10 mg)	+	+	+	+	+	+	+
Mandelamine (3 mg)	+	+	+	+	+	+	+
Novobiocin (30 mg)	+	+	+	+	+	+	+
Vancomycin (30 mg)	+	+	+	+	+	+	+
Ampicilin (10 mg)	+	+	+	+	+	+	+
Bacitracin (10i.u)	−	−	−	−	−	−	−

+++ highly sensitive, ++ moderatly sensitive,
+ slightly sensitive, − resistant

4 Results of experimental infection

Experimental infection failed to occur without damaging the fish natural barrier (Group A). Isolates 3, 5 and 7 induced mortality of all infected fish but varied in their MDT. The MDT for isolates 3 and 7 was prolonged by I/M infection and shortened by keeping the infected fish in water containing 168 mg NH_4 Cl/l (Tab. 3).

Histomorhological alternations in gill of experimentally infected animals revealed the following:

Group A:

No histopathological altrations were observed.

Group B (injured gill barrier):

Vasodilatation of the branchial blood vessel, telangectasis of capillary beds in the secondary gill lamellae, round cell infiltration between branchial epithelium and blood vessels together with overpopulation of eosinophilic granular cells (EGC) especially toward the gill arches were observed in all gills examind. Moreover, inflammatory oedema between capillary beds and epithelial sheet of the secondary lamellae which appeared with foamy cytoplasm and pyknotic nuclei were permanently seen. Hyperlastic epithelial proliferation was seen in the secondary gill leaflets with the resulting epithelial plaques formation. The superfacially located cells of the epithelial plaques commonly underwent cytoplasmic disintegration and nuclear karyolysis.

Tab. 3: Results of experimental infection of O. niloticus with F. columnaris isolates

Isolate No.	Route of infection	No. of used fish	No. of survivor fish	MDT* (hours)
1	Keeping in	3	3	0
2	water	3	3	0
3	containing	3	3	0
4	10^3	3	3	0
5	bacteria/ml	3	3	0
6		3	3	0
7		3	3	0
Control (A)	**	3	3	0
1	Installation	3	3	0
2	of 2.16×10^4	3	3	0
3	bacteria/ml	3	0	52
4	on	3	3	0
5	sacrified	3	0	160
6	gills	3	3	0
7		3	0	64
Control (B)	**	3	3	0
3	I.M.-injection of 2.16×10^4	3	0	64
7	bacteria/fish	3	0	72
Control (C)	**	3	3	0
3	Like (B) with keeping in water	3	0	26
7	containing 168 mg NH_4 Cl/ml	3	0	52
Control (D)	**	3	3	0

$$* \text{MDT} = \frac{(\text{No. of fishes died at x hours}) \cdot x + (\text{No. of fishes died at y hours}) \cdot y}{\text{Total number of died fishes}}$$

** Control groups were treated in the same manner like experimental ones with using sterile instead of bacterial suspension.

Group C (I. M. inculation):
Same as in group B, however, the proliferative changes of the epithelial lining gill lamellae were less pronounced and were localized at the tips of these lamellae.

Group D (injured gills and ammonia):
In this group, there was a marked dilatation of the branchial vessels in gill arches and lamellae together with great amount of oedematous fluid between branchial epithelium and capillary beds. Epithelium lining the secondary lamellae appeared swollen, vaculated with pyknotic nuclei and showed in some areas slight proliferative changes particulary at their attachement with primary gill filaments. A characteristic feature was the necrosis of the lamellar epithelial coat of gill lamellae.

5 Discussion

The morphological, cultural and biochemical characteristics of our 13 isolates resemble those of *Flexibacter columnaries*, as described by BUCHANON and GIBBONS (1974). Columnaris disease, caused by *F. columnaris*, is a common cause of losses among freshwater and marine fishes. Although this disease has been recorded in 44 cultured and wild species of fish, its pathogenesis is still obscure (SCHÄPERCLAUS, 1979).
This bacteria are often commensals on the gill and outer integument (BULLOCK et al., 1971; POPP, 1980). SPANGENBERG, 1975; RICHARDS et al., 1985; KUMAR et al., 1986 reported natural outbreaks of columnaris following handling stress, vitamin deficiency and ectoparasite attacks. This may explain why no isolates were able to produce the disease unless one of the natural barriers of fish body was injured.

In the case of group B (with gill injuries), there was a marked variation in the pathogenicity of the seven isolates tested (Tab. 3). While isolates 3 and 7 have relatively short MDTs of 52 and 64 hrs respectively, isolate 5 has a moderately long MDT of 160 hrs. Isolates 1, 2, 4, and 6 were not virulent. These results are similar to those of PACHA and ORDAL (1963), who detected a marked difference in the pathogenicity of 500 *F. columnaris* strains. Moreover, serological comparisons indicated differences between pathogenic and non-pathogenic *F. columnaris* strains (PACHA and PORTER, 1968).

Some authors considered a strain of *F. columnaris* to be highly virulent when it can cause the death of infected salmon within 24 hours (BUXTON and FRASERM, 1977) or 48 hours (AMEND, 1970), while low virulence is indicated by the death after several days. Similary, on the basis of MDT isolate 3 (MDT = 52 h) is considered the most virulent of those tested.

The gill pathology, death of the fishes and reisolation of *F. columnaris* following intramuscular inoculation proved, that the nature of this disease in *O. nicloticus* is systemic. However, the longer MDT of group C (intramuscular injection) than B (injured gills) after infection with isolates 3 and 7 proves the importance of the gills in the establishment and development of columnaris infection. It is therefore mentionsworthy, that *Flexibacteria spp.* were reported to have a tropism for ectodermal tissues with obscure chemical basis (MUNRO, 1982). Moreover, in acute cases of columnaris disease, the gills are the only organ with gross lesions (SNIESZKO and BULLOCK, 1976).

In naturally infected *O. niloticus*, the disease became chronic, as indicated by the occurence of excessive proliferative and necrotic changes. On other hand, the severe dilation of the branchial blood vessels, oedema, round cell infiltration, and the presence of EGC proved that the disease among experimentally infected tilapias was acute.

The dilation of branchial blood vessel and the hyperplasia of lamellar epithelium in naturally as well as in experimentally infected fishes were similar to those observed by FISH and RUCKER (1943) and WAKABAYASHI et al. (1970). The proliferative changes of the lamellar epithelium were distributed randomly and did not start at the distal ends

of the filaments, as described by DAVIS (1952). The observed necrosis of the epithelial plaques distinguishes columnaris infection from bacterial gill disease (BGD), which characterized by proliferative changes only (SNIESZKO, 1981). The proliferative epithelial plaques on the gill lamellae and necrosis of the gill tissue affect a great part of the respiratory surfaces, which could lead to impairment of gas exchange and the secretory and excretory functions of the gills with consequent moralities. This may explains the respiratory difficults among our infected *O. niloticus*.

The MDT was shortened markedly when the infected fishes with injured gills were kept in water containing ammonia (Tab. 3). This may be attributed partially to the extensive dilation of branchial vessels with the resulting extravasation of blood plasma and osmotic disbalance and partially to the stress induced by the chemical changes in the surrounding environment (PLUMB and WALTERS, 1980). Keeping tilapias with injured gills in water with high ammonia concentration may resemble what is happening in tilapia ponds during summer and autumn in Egypt. Tilapia ponds are stocked in the early spring and harvested during the autumn, when the biomass is maximum. Meanwhile parasitological examinations of cultured tilapia in the same area indicated high incidience of infection with monogentic trematodes *(Cichlidogyrus spp.)*, which reached a peak of 48 percent during the same period (ALYAN et al., 1985). Infection of tilapia with *Cichlidogyrus spp.* resulted in severe injury to the branchial blood vessels, allowing the entry of fish pathogens (FAISAL et al., 1984; FAISAL et al., 1985).

The antibiogram of our isolates revealed their sensitivity to Erythromycin, Oxytetracycline, and Chloramphenicol. This should be considered when choosing a therapy against columnaris infection.
It was also interesting to observe the resistance of tested isolates to the antibiotic, bacitracin. However, its use as a purifying agent in the isolation media require further study.

Our results highlight the implication of *Flexibacteria* in causing losses among cultured *O. niloticus*. Further studies are needed on the epizootiology of columnatis infection in the Egyptian fishes and the role of the environmental factors in the establishment of infection and determination of the disease course.

6 Summary

Flexibacter columnaris was isolated from 13 cultured *Oreochromis niloticus* showing respiratory disorders. The isolates developed typical swarming rhizoid colonies on Cytophaga agar medium. Antibiotic sensitivity test revealed the susceptility of *F. columnaris* isolates to oxytetracycline, chloramphenicol and erythromycin. A marked difference in the pathogenicity of seven tested isolates was observed: two were highly virulent, one was moderately virulent and four were avirulent. No experimental infection could be induced with the highly virulent isolates except after injuring one of the natural barries of the fish body. The severity of the disease as well as the medium death

time was shortened by keeping infected fishes with injured gills in water containing ammonia.

In naturally infected *O. niloticus*, the disease became chronic as indicated by the presence of excessive prolifrative and necrotic changes. On the other hand, severe dilation of branchial blood vessels, oedema and round cell infiltration proved that, the disease among experimentally infected tilapias was acute.

Zusammenfassung

Von 13 gezüchteten *Oreochromis niloticus*, die Atmungskrankheiten hatten, wurden *Flexibacter columnaris* isoliert. Die isolierten *F. columnaris* entwickelten typische schwärmende Rhizomkolonien auf dem Medium *Cytophaga agar*. Der antibiotische Empfindlichkeitstest zeigte die hohe Anfälligkeit der isolierten *F. columnaris* auf Oxytetracycline, Chloramphenicol und Erythromycin.

Ein auffallender Unterschied in der Pathogenität der sieben getesteten isolierten *F. columnaris* konnte beobachtet werden: Zwei waren hochgradig giftig, eine war mäßig giftig und vier waren ungiftig. Mit dem hochgradig giftigen *F. columnaris* konnte keine experimentelle Infektion induziert werden, außer nach einer Verletzung des Fisches. Wurden die infizierten Fische mit den verletzten Kiemen in Wasser mit Ammoniak gehalten, so war die Schwere der Krankheit sowie die mittlere Sterbezeit der Fische kürzer.

Bei natürlich infizierten *O. niloticus* wurde die Krankheit chronisch, angezeigt durch das Vorhandensein von exzessiven Wucherungen und abgestorbenen Teilen. Auf der anderen Seite beweisen große Ausdehnungen der Kiemenblutgefäße, der Oedema und Zellinfiltration, daß die Krankheit unter experimentell infiltrierten *O. niloticus* akut war.

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