قسم المراقبة الصحية على الاغذيـــة
كلية الطب البيطري ـ جامعة أسيوط
رئيس القسم :أ٠د/ توفيق البسيونـــي

مدي تواجد اليرسنيا والليستيريا فى أسماك المياه العذبه

يحـــيى حفنـــاوي ، صبـــاح مصطفـى*، رمضـــان رفاعى *

تم فحص خمسون عينة من الاسماك الطازجة جمعت من مدينة أسيوط والتى شملت ١٧ بلطى ١٠ قرموط ، ٩ شال ، ٩ بياض ، ٥ شلبه لتحديد مدي تواجد ميكروبات اليرسينيا والليستيريـــا فى لحوم وأمعاء هذه الاسماك ٠

دلت النتائج على وجود ميكروب اليرسينيا فى لحوم أسماك القرموط ، البياض والشلـــــبه بنسبة ١٠٪ ، ١١,١١٪ ، ٢٠٪ كما تم عزل هذا الميكروب من أمعاء أسماك البلطى ، القرمـــوط الشال ، البياض والشلبه بنسبة ٨٨ر٥٪ ، ٢٠٪ ، ١١ر١١٪ ، ٤٠٪ على التوالى ٠

بتصنيف عترات اليرسينيا المعزولة وعددها عشرة وجد أن معظمها (٧ عترات) مـــــــن نـــــوع biotype 1

بالنسبة لميكروب الليستيريا لم يستدل على وجوده فى أى من لحوم الاسماك الـــتى تـــــم دراستها ولكن عزل من أمعاء سمك الشلبه بنسبة ٤٠٪ ٠

تم مناقشة خطورة وجود هذه الميكروبات على الصحة العامة والاشتراطات الصحية الواجـــب توافرها للمحافظة على صحـــة المستهلـــك ٠

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OCCURRENCE OF YERSINIA ENTEROCOLITICA AND LISTERIA MONOCYTOGENES IN FRESH WATER FISH

(With Two Tables)

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SUMMARY

Fifty samples of several types of fresh water fish purchased at fish markets in Assiut were investigated for the presence of Y. enterocolitica and L. monocytogenes. Ten strains identified as Y. enterocolitica were isolated from both flesh and intestinal contents of all examined fish species. Seven Y. enterocolitica strains were of biotype 1, two were of biotype 4 and one was of biotype 2. Clarias lazera, Bagrus bayad and Schilbe mystus flesh revealed the presence of Y. enterocolitica in 10%, 11.11% and 20% of the examined specimens respectively. Further, Y. enterocolitica were recovered from 5.88%, 20%, 11.11%, 11.11% and 40% of the intestinal contents of Tilapia nilotica, Clarias lazera, Synodontis schall, Bagrus bayad and Schilbe mystus. L. Monocytogenes could be detected in 40% of the intestinal contents of Schilbe mystus. The public health implication of the presence of Y. enterocolitica and L. monocytogenes in fish was discussed.

INTRODUCTION

Fish are regarded generally as being more perishable than other high protein foods. The subsurface flesh of live healthy fish is considered bacteriologically sterile. The largest concentrations of microorganisms are found in the intestine, gills and slime. The numbers and types of microorganisms found on freshly caught fish are influenced by the geographical location of the catch and the season and method of harvest (POWELL, et al. 1979). The flora of fish are the products of the environments from which the fish are harvested (SHEWAN, 1971).

Fish and fish products have been associated with several human diseases (CHITTINO, 1972; BROWN and DORN, 1977). It has long been known that fish may be a vehicle of foodborne bacterial and parasitic infections in human beings (BROWN and DORN, 1977).

Yersinia enterocolitica ia a ubiquitous bacterium indigenous to the gastrointestinal tract of warm-blooded animals and is associated with human diseases. Numerous reports showed that Y. enterocolitica was frequently isolated from a wide variety of foods including raw milk, beef, pork, lamb, chicken, turkey, fish, mussels and oysters (TOMA and LAFLEUR, 1974; INOUE and KUROSE, 1975; HANNA, et al. 1976; KAPPERUD and JONSSON, 1976; SCHIEMANN and TOMA, 1978; EIXOTTO, et al. 1979).

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Although Y. enterocolitica has been isolated from fish and shellfish such as trout, oysters and mussels, information on distribution of this organism in River Nile and the potential on its survival during storage is scarce.

Listeria monocytogenes is a potential foodborne pathogen which can cause meningo-encephalitis, abortion and septicaemia (GRAY and KILLINGER, 1966; DOYLE, 1984 and RALOVICH, 1984). STATMATIN, et al. (1957) isolated L. monocytogenes from the viscera of pon-reared rainbow trout. However, the organism may be carried and spread by aquatic life (GRAY and KILLINGER, 1966).

The purpose of this study was to reveal the distribution of Y. enterocolitica and L. monocytogenes in some popular fresh water fish in Assiut City.

MATERIAL and METHODS

50 fresh fish specimens (17 Tilapia nilotica, 10 Clarias lazera, 9 Synodontis schall, 9 Bagrus bayad and 5 Schilbe mystus) were purchased from fish markets in Assiut Province.

The specimens were prepared for isolation of Yersinia enterocolitica and Listeria monocytogenes as follows: the muscle sample was taken under sterile condition after disinfection of the fish surface by rubbing with absolute alcohol and flaming. The gastrointestinal tract was carefully removed and opened for obtaining the contents.

For isolation of Y. enterocolitica the technique recommended by SPECK (1984) was followed where muscle and intestinal content samples were separately added to flasks containing Trypticase soy broth which incubated at 4°C for 14 days. Enrichments were streaked after incubation onto Cefsulodin-irgasan-novobiocin (CIN) agar plates (SCHIEMANN, 1979) which was incubated at 27°C fro 48 hours. Also, 0.5 ml of broth cultures was added to 4.5 ml of 0.5% KOH in 0.5% Nacl, then the mixture was stirred with a Vortex mixer and another CIN plate was streaked with the sample within one minute. Inoculated plates were incubated at 27°C for 2 days.

Colonies resembling Y. enterocolitica on CIN (dark red "bullseye" surrounded by transparent border, flat with smooth border) were subcultured on TSI agar slants and incubated for 2 days at 26°C. The organism was further characterized by the API 20 E (Analytab Products Inc, Plainview, NY, USA).

Biotyping of Y. enterocolitica according to Wauter's Biotype Schema cited in the Compendium of Methods for the Microbiological Examination of Foods (SPECK, 1984) was followed for classification.

Isolation of L. monocytogenes from the prepared fish muscle and intestinal contents was done by suspending them in Tryptose phosphate broth supplemented by 40 mcg/ml nalidixic acid, 30 U ml polymxin B and 10 mcg/ml tryaflavine. Inoculated broth was kept at 28°C for 5 days then one loopfull was transferred to RALOVICH, et al. medium (Tryptose agar with 5% normal horse serum) modified by HOFER (1983). Inoculated plates were incubated at 37°C for 48 hours and colonies showed a darkened central area were kept on Tryptose agar slants for confirmation. According to the procedures adopted by GRAY and KILLINGER (1966) and RALOVICH (1984), isolates giving a catalase reaction and that were motile at 21°C were examined further with API 20 S strips (Analytab products). Serological slide agglutination tests were done according to the manufacturer's on all isolates thought to be L. monocytogenes using commercially prepared Listeria O Antiserum Poly (Difco).

RESULTS

Yersinia enterocolitica occurred more frequently in the intestinal content samples (14%) than in fish flesh (6%) as presented in Table (1). Furthermore Listeria monocytogenes could be detected in one type of fish (°Schilbe mystus) where 2 out of 5 intestinal content samples proved to contain such organism.

All Y. enterocolitica isolates were typeable. The identified biotypes were 1, 2 and 4, where most of the strains (7 strains) were of biotype 1 as shown in Table (2).

DISCUSSION

Recults show that both Y. enterocolitica and L. monocytogenes were recovered from the examined fresh water fish.

Yersinia enterocolitica is one of the human pathogens that can grow at refrigeration temperature, and its presence in food is of public health concern. It has been isolated from fish and shell fish in varied percentages (TOMA and LAFLEUR, 1974; KAPPERUD and JONSSON, 1976; PEIXOTTO, et al. 1979 and HACKNEY, et al. 1980). The isolation of Y. enterocolitica biotype 1 from most of the examined samples was in accordance with that of TOMA (1973) who revealed the presence of biotype 1 Y. enterocolitica in 4 out of 17 oyster samples.

Human infection due to Y. enterocolitica have now been reported in numerous countries all over the world. There has been a steady increase in the recorded incidence of infection and in the number of countries which report this infection. In humans, the most common syndromes are gastroenteritis, mesenteric lymphadenitis and terminial ileitis; other symptoms may include polyarthritis, erythema nodosum, septicaemia and meningitis (TOMA and LAFLEUR, 1974).

Listeria monocytogenes has been recognized as a human and animal pathogen for more than 50 years; however, its prominence as a foodborne pathogen has only recently surfaced. Listeric infections in both animals and man are more prevalent than published reports indicate. However, listeric infection is not necessarily as an acute highly fatal disease but may be manifested by low-grade (GRAY, 1963 b and DOYLE, 1984).

Listeria is listed among the psychrotrophic food organisms that can grow at 3°C like Y. enterocolitica (DOYLE, 1984). The ability of the organisms to survive at low temperature in meat makes it a potential public health hazard (KHAN, et al. 1973). Also, the capability of the organism to grow at low pH nearly around 5.6, indicates that this organism is able to grow readily in the range of pH of meat and meat products (SHAHAMAT, et al. 1980 a).

However, prevention of fish and shellfish-associated illness of man as recommended by BROWN and DORN (1977) is possible by: (a) using only fish and shellfish from unpolluted waters, (b) use of proper refrigeration facilities, (c) practicing strict sanitation in processing plants and storage facrlities, (d) assuring food handlers are free of disease, (e) cooking thoroughly all fish and shellfish before eating, and (f) not handling aquatic foods when one has wounds or abrasions. Further investigation concerning the viability of Yersinia in fish is subjected for studies as part II.

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Table (1)

Isolation of Yersinia enterocolitica and Listeria monocytogenes from fresh water fish

Fish species	Number examined	Number and percentage* of positive samples			
		Y. enterocolitica		L. monocytogenes	
		Muscle	Gut	Muscle	Gut
Tilapia nilotica	17	0	1 (5.88%)	0	0
Clarias lazera	10	1 (10%)	2 (20%)	0	0
Synodontis schall	9	0	1 (11.11%)	0	0
Bagrus bayad	9	1 (11.11%)	1 (11.11%)	0	0
Schilbe mystus	5	1 (20%)	2 (40%)	0	0
Total	50	3 (6%)	7 (14%)	0	2 (4%)

* According to species.

Table (2)
Biotypes of Yersinia enterocolitica isolated from fresh water fish

Fish species	Wauters biotypes		
	1	2	4
Tilapia nilotica	1	0	0
Clarias lazera	1	1	1
Synodontis schall	2	0	2
Bagrus bayad	1	0	0
Schilbe mystus	2	0	0
Total	7	1	2