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Epizootics of *Lernaea cyprinacea* (Copepoda: Lernaeidae) in imported cyprinids to Egypt

Epidemie bei den in Ägypten eingeführten Cypriniden verursacht durch *Lernaea cyprinacea*

by M. Faisal[1], M. el-S. Easa[2], S. I. Shalaby[3] and M. M. Ibrahim[4]

1 Introduction

With the ever-increasing expansion in aquaculture in Egypt, genetically-selected stocks of cyprinids (e.g. common, grass, silver and bighead carp) were imported from different parts of the world (FAISAL, 1986). This practice, however, is associated with the introduction of fish pathogens that might settle under the subtropical mild environment thus constituting a potential hazard to local fish breeds (REICHENBACH-KLINKE, 1980). In a previous study (FAISAL and EASA, 1987), we reported the isolation of a capsulated Pseudomonas spp. from septicaemic silver carp, *Hypophthalmichthys molitrix*, directly following their import, which showed high pathogenicity to local fish breeds by experimental infection.

In the present report, we describe three epizootics among the imported broad stocks of common *Cyprimus carpio* and grass carp *Ctenopharyngodon idella* and their progeny by a pathogenic copepod, *Lernaea cyprinacea L.*, that is known to cause high losses in cultured fish in different parts of the world (CHECHINA, 1954; KABATA, 1970; KÖRTING, 1985 and TIMMONS and HEMMSTREET, 1980).

[1] Dr. M. Faisal, Dept. of Avian and Aquatic Animal Medicine, Faculty of Veterinary Medicine, University of Alexandria, Egypt
[2] M. el-S. Easa, Dept. of Parasitology and Pathology, Faculty of Veterinary Medicine, University of Cairo, Egypt
[3] S. I. Shalaby, Dept. of Parasitology and Animal Diseases, National Research Centre, Dokki, Egypt
[4] M. M. Ibrahim, Fish Deseases Lab, Animal Health Research Institute, Dokki, Egypt.

2 Material and Methods

2.1 Parasitological examination

Collected copepods were fixed in 70% alcohol glycerine and permanent mounts were prepared, either by being passed in ascending grades of Alcohol, cleared in Xylol, then mounted in Canada balsam or cleared in lactophenol and mounted in glycerine gelatine. Twenty to thirty parasites from each species and fish farm were measured. Drawings were made with the aid of camera lucida.

From skin lesions, wet smears were prepared and examined for the presence of other parasites under the light microscope according to the method described elsewhere (FAISAL, 1985).

2.2 Histopathological examination

Skin and underlying muscles of affected areas were fixed in 10% formaline buffered saline, embedded in paraffin and stained with Haematoxylin and Eosin (H + E) according to the methods described by HIBIYA (1982).

2.3 Effect of infection on fish weight

The fork length (L.±0.1 cm) and weight (W.±0.01 g) were determined in infected and noninfected common carp fingerlings. Statistical analysis including the regression equation and coefficient between uninfected and infected (at different intensities of parasitism) living in the same pond was conducted according to the method described by WEBER (1972).

2.4 Treatment and control

In the case of broad fish, the copepods were removed mechanically, and fish were bathed in a freshly prepared solution of potassium permanganate at a concentration of 25 g/m^3 for 30 minutes as recommended by SCHÄPERCLAUS (1979) in fiberglass tanks (1 m^3 capacity) and then transferred to another earthen pond or cement raceway supplied with filtered water. Fish showing perforation or ulcerations were quarantined in separate, aereated 100 m^3 raceways and were given, besides the above-mentioned bath, specially prepared high protein (40%) pellets that contained oxytetracycline (50 mg/kg living body weight) to prevent secondary infection.

On the other hand, carp fingerlings (infected and noninfected of the same pond) were condemned, and the ponds were disinfected using quick lime in the concentration of 0.25–0.5 kg/m^2 as recommended by REICHENBACH-KLINKE (1980).

3 Results

3.1 Clinical studies

In the past two years, 3 epizootic copepod infections to the skin of cyprinids were re-

ported to the Laboratory of Fish Diseases, Dept. of Avian and Aquatic Animal Medicine, Faculty of Veterinary Medicine, University of Alexandria. The first one was among 4-year old adult common carp *Cyprinus carpio* and grass carp *Ctenopharyngodon idella* that were imported 2 days before the notification. 43 out of 180 (23.9%) grass carp and 31 out of 190 (16.3%) common carp showed the attachment of worm-like graygreenish copepods that were focally distributed along both sides of the body, with preference to the pectoral and tail fins. The number of copepods/fish ranged in grass carp 10–20 (average 13.5) and in common carp 16–25 (average 19.7). Moreover, in some grass carp, the parasitic elements were completely covered by haemorrhagic nodules that emerged from underneath the scales (Fig. 1). In a ripe female common carp, a large perforated ulcer (Fig. 2) was observed with 5 parasites in the center and fragments of the ovary containing eggs shredded out of the body. Mortalities of 10% were observed in both affected species. No copepod infection was observed in silver or bighead carp in the same pond.

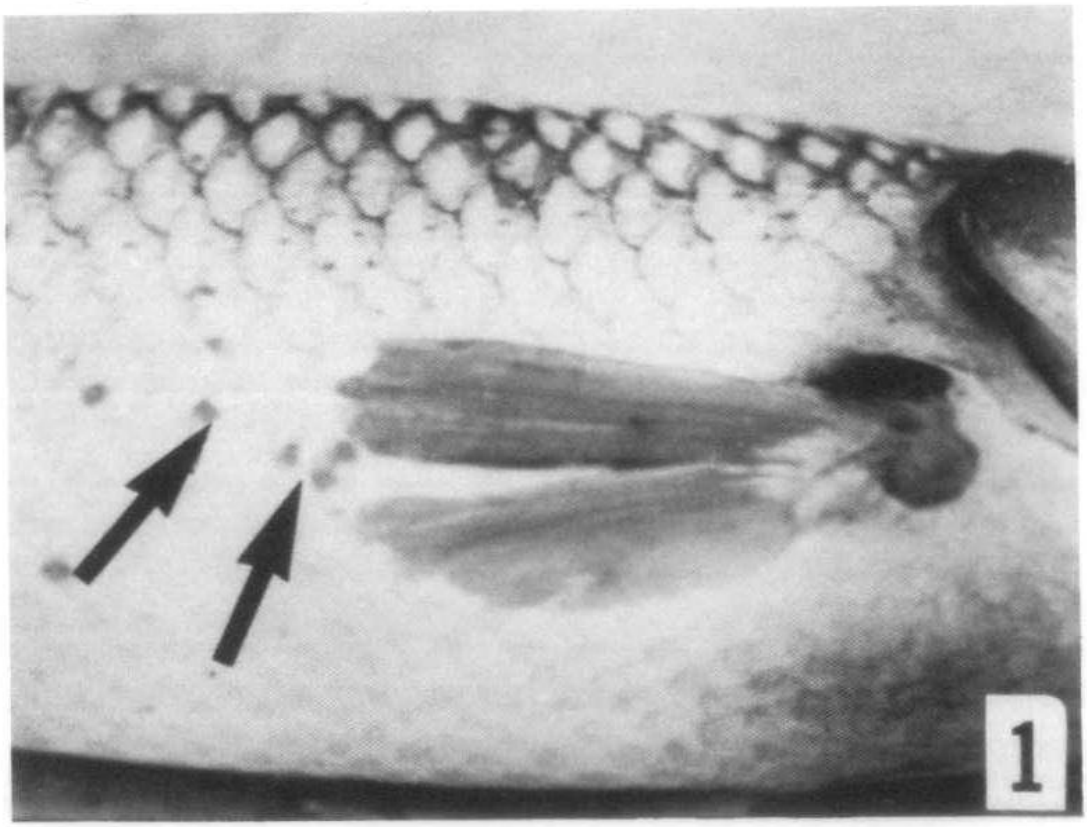

Fig. 1: Grass carp *(Ctenophyryngodon idella)* covered by haemorrhagic nodules

The second outbreak was observed among one-year-old common carp fingerlings (35 g ± 4, 11 cm ± 1) that were stocked in 2 earthen ponds (1000 m² each) in a fish farm in Sharkiah (Northeast Egypt). There is a history of recurrent incidence of this infection in both *C. carpio* broad stock and fingerlings in the same hatchery since the import of the original stock 3 years ago. The parasites were attached to the skin and underlying tissue in a focal manner all over the body surface, particularly at the tail (Fig. 3).

Fig. 2: Common carp 'female' *Cyprinus caripio)* with perforated ulcer.

Fig. 3: *C. carpio* broad stocks and fingerlings attached by parasites to the skin, particularly at the tail

Small 1 mm reddish ulcers that sometimes coalesced to larger ones were observed at the flank region and occasionally developed into perforated ulcers with the parasite attached to the liver. In rare instances, the parasite attached to the eyeball or to the roof of the mouth (Fig. 4). The copepod infected 76% of the stock and the number of parasites/fish ranged between 10–17 and mortality reached 20%.

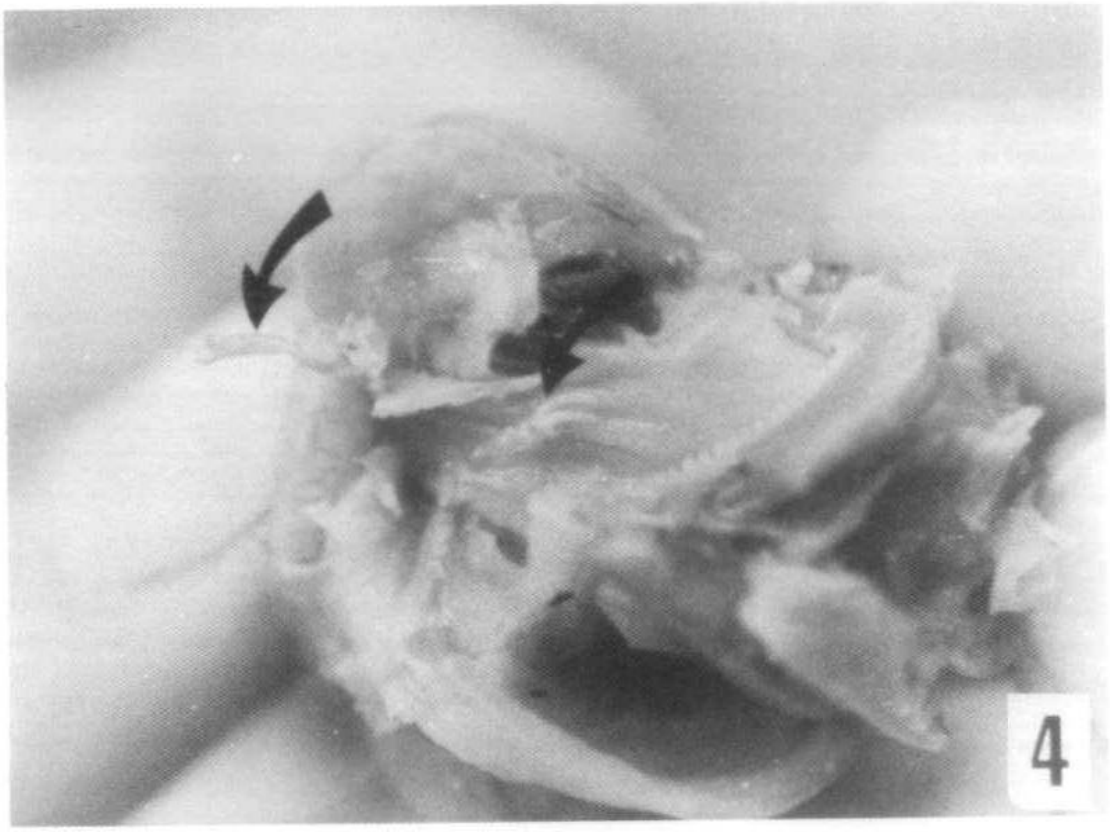

Fig. 4: Parasites occasionally attached to the eyeball or to the roof of the mouth.

The third outbreak was observed in the broad stock of grass carp in a fish hatchery at *Suez Canal University*, that was kept in cement raceways (capacity 300 m^3) to produce fingerlings for the purpose of combating weeds in the River Nile and its canals. Again, there was a history of recurrent incidence following spawning seasons (May-June) since their import. The skin lesions were similar to those described before. The infection rate was 60% (120 out of 200) and the number of parasites/fish ranged between 1–17 (average 3.5). Also, there were erosions in the tail and fins of affected fish.

3.2 Parasite description (*c.f. Fig. 5*)

The parasite appeared as a spindle-like structure that measured 6–22 mm (average 13.8 mm) in length and 0.18 mm in width. The head was represented by a round knob projecting from the anterior margin of the cephalothorax with a deeply buried triparti-

te eye near the center of the dorsal surface that measured 0.05 mm in diameter. A pair of conical, soft, and branched horns were detected on the lateral margins of the cephalothorax and each measured 0.25 × 0.12 mm. The neck was soft, slender and enlarged gradually into a cylindrical trunk. The abdomen was distinctly trisegmented and terminated in a pair of caudal rami. Two external elongated egg sacs (0.50x 0.04 mm) appeared, containing rounded, yellowish- brown eggs with thin, transparent shells. The eggs were arranged in more than one row in each sac, and each egg measured 0.08–0.1 mm in diameter. Four pairs of very small legs were seen, one just behind the head and the others at increasing distances posteriorly.

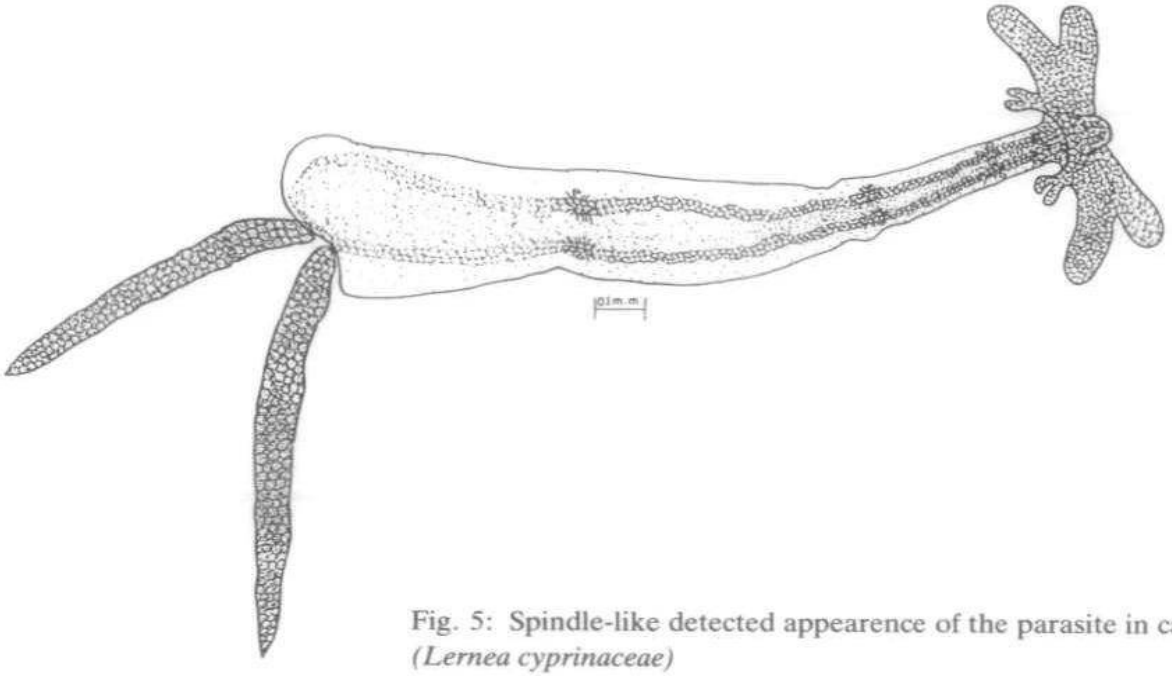

Fig. 5: Spindle-like detected appearence of the parasite in carp fishes *(Lernea cyprinaceae)*

3.3 Histopathological lesions

At the point of the parasite settlement in the skin and underlying tissues, extensive damage was observed in the form of sloughing of most of the epidermis and sometimes the dermis. The copepod anchor was surrounded by a fibrous connective tissue capsule and inflammatory cells, as well as melanin-carrying cells. In the area around the capsule, there was extensive dilation of blood vessels and occasional hemorrhages. The epithelial lining of the epidermal layer of affected skin and fins showed hypertrophy and hyperplasia (Fig. 6) that sometimes rose above the surface and formed clear outgrowths. In areas where the copepod penetrated deeply, with the consequent formation of cup-shaped depressions or button-shaped ulcers, there was complete absence of both the epidermis and dermis. The remaining epidermal cells appeared swollen and the dermis was infiltrated with inflammatory and melanin-carrying cells. At the margin of the ulcerated area, the epidermal cells appeared hypertrophied and hyperplasic with their nuclei stained dark. A clear edema was apparent in the underlying hypodermic and muscular layers which showed leukocytic infiltration and dilated blood vessels.

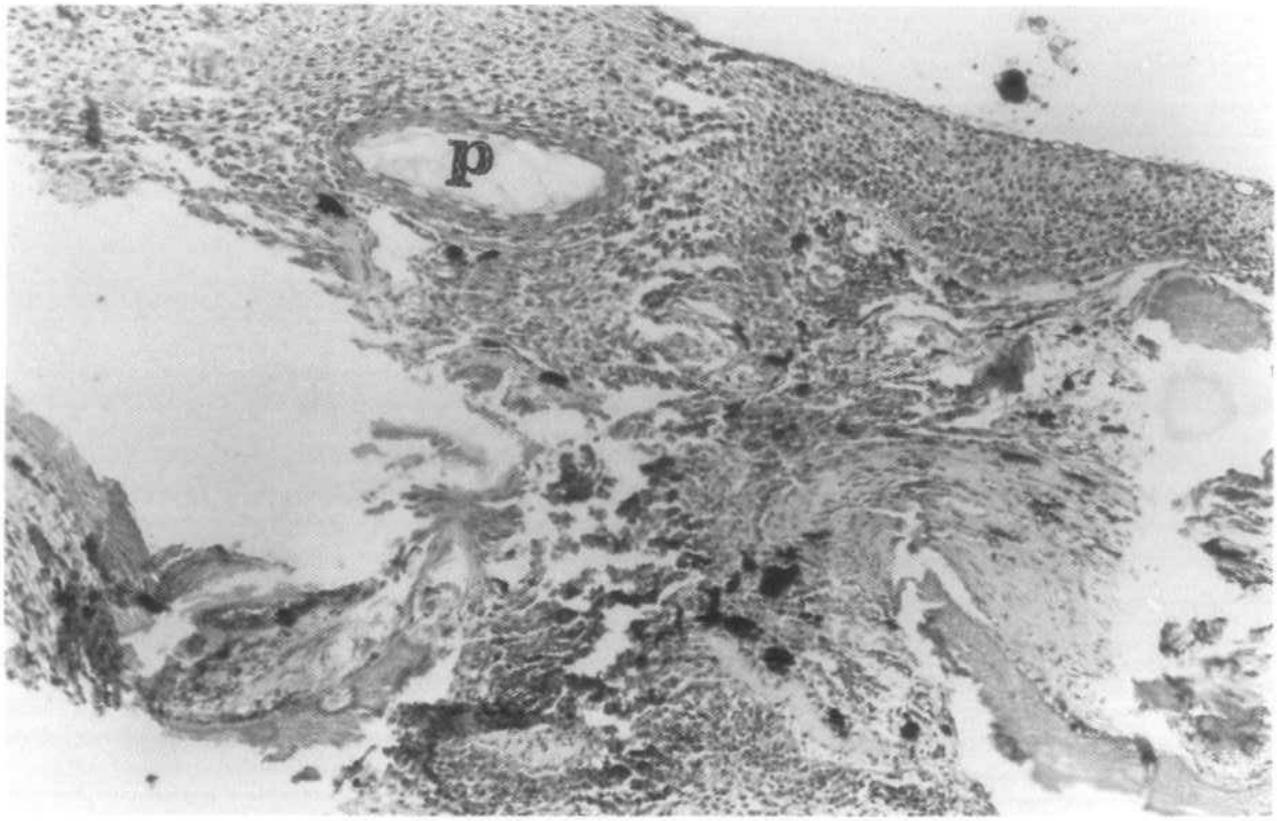

Fig. 6: Hypertrophy and hyperplasia of the affected skin in carp fishes (× 400).

The necrotic changes extended to the muscular tissue which suffered severe mechanical destruction even in the absence of parasitic elements. Myomalacia, myophagia and a considerable number of inflammatory cells which were mainly lymphocytes, macrophages and plasma cells were noted. Moreover, many fibrous connective tissue proliferation with or without parasitic elements were abundant within the muscular tissue.

3.4 Effect of Lernea cyprinacea on the length-weight relationship of common carp fingerlings

Results of regression analysis on common carp fingerlings, uninfected and infected with different numbers of *L. cyprinacea*, are presented in Tab. 1. Differences between regression lines for uninfected and infected fish with ≥ 3 Lernea/fish were statistically significant. Moreover, a significant difference between regression coefficients was recorded only in the case of infection with ≥ 6 Lerneas/fish. Results indicate, therefore, that very light infection with 1–2 parasites/fish have negligible effect on the fish while when the number of parasites/fish ≥, a significant weight reduction took place.

3.5 Treatment efficacy

Potassium permanganate proved to be effective against *L. cyprinacea*. Following an observation period of one year post treatment, no infection recurrence was observed. Concerning the quarantined fish, perforations and ulcerations healed within a month and were returned back to the earthen ponds.

Tab. 1: Common carp uninfected and infectedwith *Lernea cyprinaceae:* logarithmic length- weight regressionand significance tests.

Degree of Infection	No. of fish in sample	Regression Equation	Significance Tests			
			Regression lines		Slopes	
			Variance Ratio, F	Probability, P	t	Probability, P
Uninfected	37	log W=3.082 log L=2.123	—	—	—	—
Infected (1–2 L/f)	49	log W=3.185 log L=2.242	1.80	P>0.05 (ns)	1.568	P>0.1 (ns)
Infected (3–5 L/f)	43	log W=3.045 log L=2.158	31.83	P<0.001 (s)	0.240	P>0.1 (ns)
Infected (≥6 L/f)	79	log W=2.437 log L=1.407	26.17	P<0.001 (s)	7.186	P>0.001

L/f = Lernea/fish; ns = not significant; s = significant

4 Discussion

The morphological characteristics of the copepod recovered from the 3 epizootics indicate its similarity to *Lernaea cyprinacea* (Family Lernaeidae) as described in YAMAGUTI (1963). *L. cyprinacea* was reported to induce economic losses not only among cultured fish (DOGIEL et al., 1958; KÖRTING et al., 1985; REICHENBACH-KLINKE, 1980 and TIDD, 1934) but also in wildfish dwelling in rivers (SCHÄPERCLAUS, 1979) and even large water reservoirs (TIMMONS and HEMSTREET, 1980). Hence, we stress the necessity of strict veterinary measures during shipment of live fish between different countries, particularly in tropical and sub-tropical areas, where the parasite can settle and produce up to 11 generations/year (YASHOUV, 1959) in comparison to 2 generations/year in Europe (GRABDA, 1963).

Attachment of the parasite to its susceptible fish host occurs when the cyclopoid female penetrates the host surface, where it elongates and undergoes metamorphosis of the cephalic region to produce a branched anchor process which is embedded in the host skin and feeds on the blood and other tissue fluids (GRABDA, 1963; ROGERS, 1968).

SHIELDS and GOODE (1978) described the formation of thickened whorls of hyperplastic epithelium and fibrous tissue around the parasite in the skin of goldfish, *Carassius auratus* (L.), infected with *Lernaea cyprinacea* as a rejection response of the host to the parasite. Similar response was found in the present study. Moreover, we found inflammatory reactions and occasionally hemorrhage around the parasitic elements, which indicated severe tissue reaction. This might have resulted from continuous movement of the parasite. Moreover, calcification around the anterror part of the parasite occasionally occurs (KHALIFA and POST, 1976).

In carp fingerlings, a significant reduction in infected fish was observed as the number of copepods/fish exceeded 2. In this respect, SUKHENKO (1964) stated that as few as 2–3 adult parasites could kill affected fish. This could be attributed primarily to the permanent feeding habit of *L. cyprinacea* on blood and tissue fluids; however, other contributory factors should not be neglected. The ongoing damage of dermis and epidermis together with the connective tissue formation around the anchors, in addition to the observed subcutaneous edema, would certainly impair some skin functions like osmoregulation and excretion, thus disturbing the homeostasis of the affected fish (LAGLER, 1979). The attachment of *L. cyprinacea* to unusual sites like eyeballs, buccal cavity, liver and gonads thus impairing food uptake, normal swimming movements, and organ function can also contribute to this condition. Nevertheless, injured skin barriers allow the opportunistic bacteria to invade the body and induce chronic or acute bacterial infections (FAISAL and GHONEIM, 1985; FAISAL and POPP, 1987a, b).

We recommend the condemnation of these infected fingerlings and disinfection of the earthen ponds with Quick lime in the concentration recommended by SCHÄPERCLAUS (1979) due to the expected growth retardation as well as the possible spread of the parasite to other farms via purchase of these fingerlings from the hatcheries.

On the other hand, treatment of broad stocks with potassium permanganate baths and disinfection of the pond was quite sufficient to get rid of attached parasites as detected by their posttreatment screening for one year. In spite of the narrow safety index of this chemical, its potent anti-Lernaea effect convinced some authors to recommend its periodical use for prophylaxis against copepod infections (SUKHENKO, 1964; WEN-YING et al., 1963); however, precautions against possible fish toxicity should be undertaken.

In the present study, we report the incrimination of *L. cyprinacea* in 3 epizootics in imported cypriniid flocks in Egypt for the first time. The presence of this infection in hatcheries raises the possibility of its further spread, thus needing continuous prophylactic measures. Whether or not this parasite settled in other farms or in the Nile water systems is still unknown and needs continuous investigations and control.

The present study and our previous report (FAISAL and EASA, 1987) raise the possibility of introducing new fish pathogens through fish import. The authors hope that traffic of live fish, particularly to tropical and subtropical countries will be governed by regulatory laws and be inspected by specialized veterinarians.

5 Summary

In the past few years, many countries of subtropical and tropical areas have imported broadstocks of rapidly growing fish to establish aquaculture projects. However, this procedure can be associated with some ecobiological changes.

In Egypt, where serious efforts were directed at increasing fish production, broad stocks of the family Cyprinidae were imported in the last three years from different parts of the world. However, our laboratory was notified of the presence of 3 epizootics among these fishes in different Egyptian hatcheries. Parasitological examination revealed the dangerous copepod *Lernaea cyprinacea* as the etiological agent. *L. cyprinacea* induced a severe degenerative and necrotic changes in the skin and muscles of affected fish, perforated the abdominal cavity, attached to the eyeball, liver, and buccal cavity, and induced growth retardation in fingerlings. Hence, we stress the necessity of strict veterinary measures during live fish shipment between different countries, particularly in tropical and subtropical areas, where pathogens might constitute a potential hazard to local fish breeds.

Zusammenfassung

In den letzten Jahren wurden in verschiedene sowohl subtropische als auch tropische Länder Laichfische der schnell wachsenden Arten eingeführt um Aquakulturprojekte durchzuführen. Das könnte jedoch zu einigen Veränderungen der biologischen Elemente des Ökosystems führen.

In Ägypten, wo ernsthafte Anstrengungen unternommen wurden, um die Fischproduktion zu erhöhen, wurde in den letzten drei Jahren Laichfische der Familie Cyprinidae aus verschiedenen Teilen der Welt importiert. Uns wurde von Epidemien bei diesen Fischen in drei ägyptischen Fischbruthäusern berichtet. Nach parasitologischen Untersuchungen konnte der gefährliche Copepod *Lernaea cyprinacea* als Erreger identifiziert werden. *L. cyprinacea* hat zu ausgedehnter Degeneration der Haut und Muskulatur, Durchlöcherung der Bauchhöhle und zu Verlangsamung des Wachstums bei Karpfenbrütlingen geführt. Im übrigen heftete sich dieser an die Augen, an die Leber und an die Wand der Mundhöhle.

Aus diesen Gründen ist die Einhaltung strenger tierärztlicher Kontrollen während des Transportes lebender Fische unentbehrlich, zumal in den tropischen und subtropischen Zonen, wo die Krankheitserreger, die mit den Importfischen mitgeschleppt wurden, die einheimischen Fischarten gefährden können.

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