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AN OUTBREAK OF A DISEASE OF FARMED EEL (ANGUILLA ANGUILLA) DUE TO AEROMONAS HYDROPHILA İN TURKEY: HİSTOPATHOLOGICAL AND BACTERIOLOGICAL STUDIES

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Yurdumuz kültür yılan balıklarında (Anguilla anguilla) etkeni Aeromonas hydrophila olan hastalığın histopatolojik ve bakteriyolojik yönden incelenmesi.

Özet: *Bu çalışmada Eskişehir, Çifteler Sakaryabaşı Balık üretim ve Araştırma İstasyonunda değişik rasyonlarla beslenen yılan balıklarında (Anguilla anguilla), Aeromonas hydrophila'nın neden olduğu hastalık, bakteriyolojik ve histopatolojik yönden incelenmiştir.*

Klinik ve histopatolojik bulgularda yaygın deri ülseri, pullarda dökülme, uzun süreli olaylarda ise deri altındaki kas tabakasına kadar ilerleyen etkenin miyopati ve miyofajiye neden olduğu saptanmıştır.

Summary: *The present study describes an outbreak of Aeromonas hydrophila infection among the eel (Anguilla anguilla) at Çifteler-Sakaryabaşı Fish Breeding and Research Station in Eskişehir-Turkey.*

The main clinical and histopathological feature of the diseases was the development of extensive skin ulcere, move out of scales and in longer standing lesions the myopathy and myophagia of underlying musculature.

Introduction

Bacterial fish disease caused by members of the genera *Aeromonas* and *Pseudomonas* are very common among fishes. The great epizootics described in Europea (5). The disease is common in warmwater pondfishes in the United States in rainbow trout *(Salmo gairdneri)* in the Western States and in northern pike *(Esox lucius)* in Canada (12).

The disease was characterized by ulceration of the skin and extending down to penetrate the underlying musculature (2, 3, 6, 10, 1)).

Materials and Methods

Live specimens of eels *(Angilla anguilla)* with characteristic lesions were obtained from the Çifteler-Sakaryabaşı Fish Breeding and

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Research Station. Measured 16,60 + 0,17 cm., in length and 12,38 + 0,33 g., in weight. Affected live fish were brought to the Unit of Fisheries and Fish Disease in Ankara for histopathological and bacteriological studies.

For patological investigation at 10 per cent formalin fixed and paraffin embedded samples of skin, kidney, gills, liver, spleen from affected fish were cut at 5 microns and stained with heamotoxylin and eosin.

For bacteriological examination, the sample was taken aseptically from the lesion and from kidney, liver, heart, blood and spleen. It was streaked on the blood agar (added 10 per cent sheep blood) and frunculosis agar (8). Smears from these tissues were also made and stained by Gram's method. Biochemical examination of the isolates were carried out according to the technique of Osbaldiston (7). The sensitivity disc was used in the therapeutic use of antibiotics.

Results

Gross Pathology

The disease was characterized by external lesions on both flanks varying in severity from small areas of scale loss to large ulcers which extending down to penetrate the underlying musculature. Haemorrhage on the fins were also present. Internally numerous petechial haemorrhages observed on the walls of intestinal tract.

Histopathology

Gill: Gill flament were lost their morphologic features Hypertrophic epithelial cells of the seconder gill lamellar were occupied with bacteria (Fig. 1) and loss of cellular outlines and phyknosis of necrotic cells were present.

Liver: Necrosis of hepatic perenchymall cells was first evident. Surrounding paranchymall cells of vena centralis were lost their cell membrane and nuclei, and many bacteria were seen around them (Fig. 2).

Kidney: Loss of cellular outlines and nuclei of necrotic kidney tubules were present. The normally distributed reticuloendothelial tissue was spread through the kidney tubules, but some have lost their nuclei. Many bacteria were seen either within the reticuloendothelial tissue or around the tubules (Fig. 3).

Figure 1. Necrotic gill epithelial cells with *A.hydrophila* (arrowed) H.E. × 375 Nekrotik solungaç epithelial hücreleri ve *A.hydrophila* (okla işaretli).

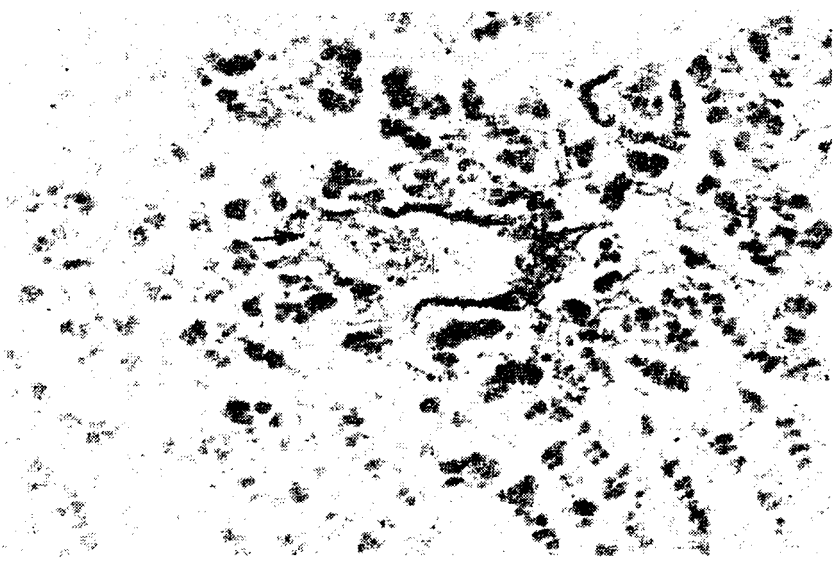

Figure 2. Hepatic parenchymal cells with loss of cellular outlines and nuclear staining and some bacteria around the cells (arrowed). H.E. x 375 Hücre zarı kaybolmuş ve çekirdekleri iyi boya almayan karaciğer paranşima hücreleri çevresinde yer alan bakteriler (okla işaretli).

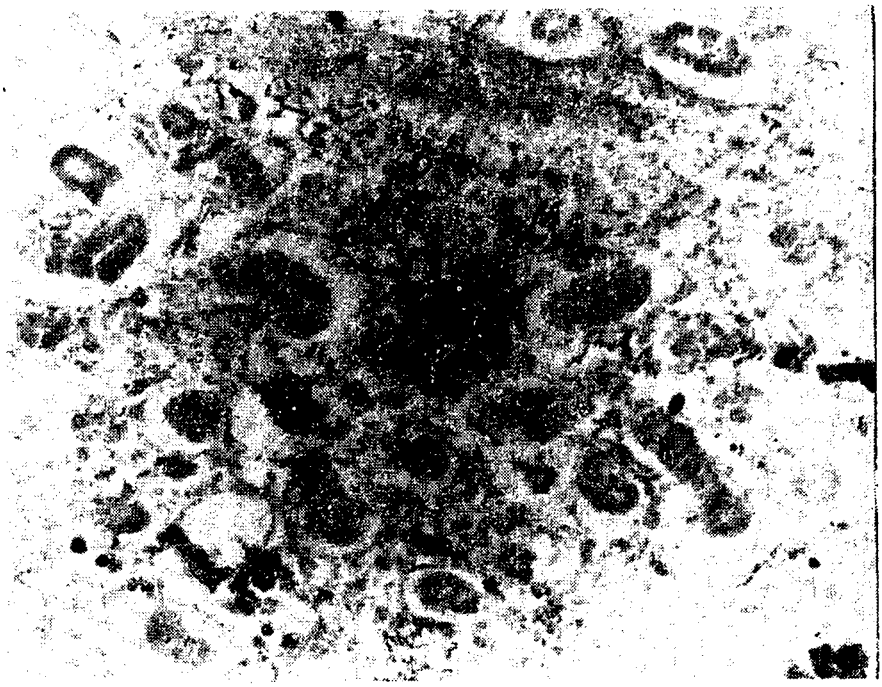

Figure 3. Melanin cells (M) and bacteria (B) within the necrotic reticuloendothelial tissue and kidney tubules. H.E. × 250 Nekrotik retiküloendotelial doku ve böbrek tübülleri arasında yer alan melanin hücreleri (M) ve bakteriler (B).

Spleen: The lymphoid tissue of the white pulp was necrotic and the bacteria were not much spread throughout the necrotic cells.

Skin: The lesions were varied depending on the stage of the infection. The edge of the ulcer showed haemorrhages and leucocytic infiltration. In chronic skin lesions the ulcer had extended to depth into the myotomal muscle fibres. Musculatire showed myopathy and some bacteria were seen around and within the muscle bundles (myophagia). At this stage, leucocyte infiltration had invaded among the capillary blood vessels of the muscle fibres.

Bacteriology

Smears from the spleen, liver, kidney and heart of the infected fish were showed Gram negative, rod shape bacteria. From the visceral organs and blood of the infected fish, circular with 1-2 mm., in diameter, regular bordered, pigmented and spreading colonies were isolated and characterized as a strain of *Aeromonas hydrophila* (formerly *A. liquefaciens)* based on the results of biochemical reacti-

ons. The results of the biochemical tests and the sensitivity test are shown in (Table 1,2).

Table 1. Biochemical reactions of *A.hydrophila*

Tests	Reactions
Motility	+
Gram's reaction	—
Hemolysis	—
Lactose	—
Glucose	—
Maltose	+
Galactose	+
Saccarose	—
Arabinose	—
Laevulose	+
Raffinose	—
Inositol	+
H_2S	—
Voges-Preskauer reaction	—
Methyl-red	+
Indole	—
Cytochrome-Oxidase	+
Oxidation-Fermentation (O/F)	F

Discussion

Clinical signs observed in the present outbreak were similar to those described elsewhere for haemorrhagic septicemia except that the severe necrosis and many bacteria were seen either within the necrotic cells or around them described by Reynolds *et al* (9) in goldfish *(Carassius auratus)* were not seen in this study.

Table 2. Reaction to antibiotics

Antibiotics	Reactions
Chloramphenicol	Sensitive
Nitrofunton	Slight sensitive
Chlortetracycline	Slight Sensitive

The lesion varied depending on the stage of the infection. Histopathological changes in the dermis and Stratum spongiosum similar to those described for *Pseudomonas* infection in eel (1) with ulcer, loss of scales, marked hyperemia, minor haemorrgahes and leucocytic infiltration. In longer standing skin lesions the underlying musculature showed myopathy and myophagia.

Natural epizootics in fish population are not uncommen but most of Gram negative bacteria unable to act as primary pathogen for fish. *Aeromonas* and *Pseudomonas* are usually pathogenic under st-

ress-free conditions. Mortality in the spawning period has also been reported in brown trout *(Salmo trutta)* by Thorpe and Roberts (13).

Aeromonas hydrophila has been identified as a true fish pathogen (4). The major pyogenic component of Gram negative bacteria is though to be a lipopolysaccharide constituent of the cell wall, referred to as endotoxin (11). This bacteria endotoxin is presumably the exogenous pyogen present in *Aeromonas hydrophila* which initiates the febrile response in fishes and other animals (9).

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