Vol. 1: 191–196, 1986 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published October 15

Outbreak of liver-cell carcinoma among saltwater-reared rainbow trout *Salmo gairdneri* in Denmark

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ABSTRACT: An outbreak of liver-cell carcinoma, involving at least 50 000 saltwater-reared rainbow trout *Salmo gairdneri* in 5 Danish marine trout farms, was associated with aflatoxin-contaminated feed from a single feed producer. Light and electron microscopical examination confirmed the diagnosis. Epidemiological aspects of the problem are considered and discussed.

INTRODUCTION

When commercial trout food pellets were introduced in the late 1950s, liver-cell carcinoma among cultured rainbow trout *Salmo gairdneri* became widespread (Wood & Larson 1961, Wales 1970). The disease was associated with cottonseed meal in the feed (Wolf & Jackson 1963). Later, aflatoxins were shown to possess hepatocarcinogenic activity in rainbow trout (Halver 1965), and outbreaks of liver-cell carcinoma in cultured rainbow trout were associated with feed contaminated with aflatoxin (Sinnhuber et al. 1965, Sinnhuber 1967, Jackson et al. 1968). This toxin is frequently present in cottonseed products (Jones 1977).

Aflatoxins are fungal metabolites produced by *Aspergillus* spp. and comprise a group of related compounds possessing highly hepatocarcinogenic activity in several animal species (Jones 1977, Patterson 1977). Rainbow trout are extremely susceptible to aflatoxins (Jackson et al. 1968).

Since the widespread outbreaks in the late 1950s and the beginning of the 1960s, several occurrences of liver-cell carcinoma in cultured rainbow trout have been reported (Wunder 1971, Roald & Håstein 1979, Majeed et al. 1984), but the disease had become more sporadic. Apparently, producers of trout feed had recognized the risk of using cottonseed meal.

Recently, an outbreak of liver-cell carcinoma among saltwater-reared rainbow trout, in 5 Danish marine fish farms, was associated with cottonseed meal containing feed, contaminated with aflatoxin. The present report concerns the detection and epidemiological aspects of this outbreak.

MATERIAL AND METHODS

Prevalence of liver-cell carcinoma. Prevalence was estimated at slaughter. From each of 3 marine trout farms (I, II, V), 200 to 300 random livers were inspected for the presence of macroscopical changes.

Marine trout farms. In autumn 1983, Marine Trout Farm I purchased 6 to 7 mo old rainbow trout from freshwater farms. The trout were reared, utilizing cooling water from a power plant, until slaughter approximately 15 mo later. Marine Trout Farms II, III, IV and V received 2 yr old rainbow trout from freshwater farms in spring 1985. These trout were held and fed in net cages for 6 to 7 mo and then slaughtered. Number and origin of fish purchased by marine trout farms, as well as the source of feed were recorded.

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0177-5103/86/0001/0191/$ 05.00

Feed. Information was collected on feed composition of trout pellets produced by different fodder mills.

Analysis for aflatoxins in trout feed and trout livers. Feed produced by Fodder Mill A in 1984 (2 samples) and 1985 (1 sample) and 13 nodular or cystic trout livers from Marine Trout Farm V were examined. After homogenization, extracts were prepared and analysed (Stubblefield et al. 1982, Stoloff & Scott 1984).

Histology and electronmicroscopy. Tissue samples for light and electron microscopical examination were taken at Marine Trout Farm V. Samples of 8 trout livers exhibiting nodular swelling and 4 trout livers without macroscopical changes were fixed in chilled 10 % buffered formalin and processed for histology. Tissue blocks from liver nodules of 8 fish were immersion-fixed in 70 % Karnovsky solution. The blocks were postfixed in 1 % osmium tetroxide and finally embedded in Epon. Ultrathin sections were cut with a LKB Ultrotome equipped with a diamond knife. The sections were stained with uranyl acetate and lead citrate in a LKB 2168 Ultrostainer and examined in a Phillips 201 electron microscope.

RESULTS

Occurrence of liver-cell carcinoma in marine trout farms, related to trout origin and feed source, is shown in Table 1. Marine Trout Farm I received trout from 3

Fig. 1. *Salmo gairdneri.* Macroscopical photo (× 1.2) of liver with carcinomas and haemorrhagic areas

Table 1. *Salmo gairdneri.* Epidemiological aspects of liver-cell carcinoma in saltwater-reared fish

Marine Trout Farm	Origin of fish received*	Number of fish received	Source of feed in fresh water farm**	Source of feed in marine farm**	Occurrence of liver-cell carcinoma in marine farm***
	1	6 000	A	B	+
I	2	4 000	A	B	+ (60 %)
	3	4 000	B	A	+ (20 %)
	1	24 000	A	B	+ (20 %)
II	4	16 500	E	B	–
	5	19 500	C	B	–
	6	13 000	A	E	+
III	7	9 000	D	E	–
	8	2 000	C	E	–
IV	9	23 000	A	A + B	+
	10	7 000	B	A + B	–
V	6	70 000	A	A + B	+ (60 %)
	11	75 000	C	A + B	–

* Marine trout farms received fish from 11 freshwater farms, numbered 1 to 11
** Trout pellets were produced by 5 fodder mills, designated A to D
*** Prevalence of liver-cell carcinoma, in brackets, was recorded at 3 marine trout farms

freshwater farms designated 1, 2 and 3. Two of these freshwater farms (1 and 2) had been using feed produced by Fodder Mill A in 1984. Trout from Freshwater Farm 3 were raised on B feed in freshwater and given A feed at Marine Trout Farm I in 1983, 1984 and 1985. All affected fish from Marine Trout Farms II, III, IV and V had been raised on trout feed purchased from Fodder Mill A at freshwater farms in 1983 and 1984.

One of 5 different fodder mills (Fodder Mill A) had been using cottonseed meal for production of trout feed from 1978 until January 1985. Analysis of feed produced by Fodder Mill A in 1984 showed a concentration of ca 2 µg aflatoxin B_1 kg^{-1} feed. Aflatoxins were not detected in feed produced by this fodder mill in 1985. Aflatoxins were not found in the livers submitted for analysis.

At gross examination, 8 livers exhibited nodules ranging from 1 or 2 mm to 2 or 3 cm in diameter (Fig. 1). The nodules were either light yellow or translucent greyish white; the latter often represented the smallest nodules.

Fig. 2. *Salmo gairdneri*. Liver from individual with liver-cell carcinoma. Note trabecular growth pattern. H-E, × 184

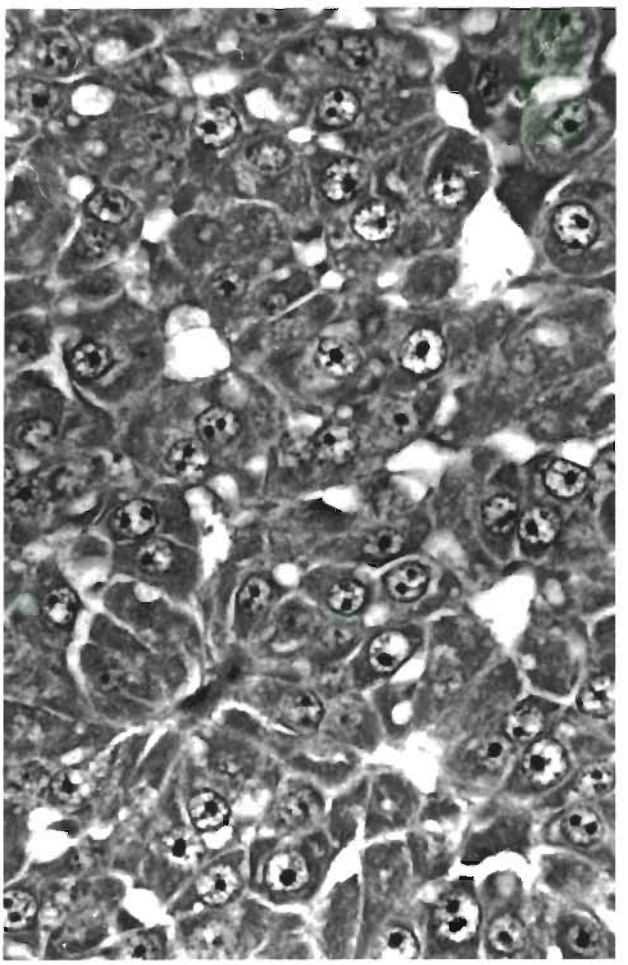

Fig. 3. *Salmo gairdneri*. Same liver as in Fig. 2. Neoplastic cells are pleomorphic and often contain 2 nucleoli. H-E, × 460

Histologically 9 livers exhibited varying degrees of liver-cell carcinoma. The growth pattern of the neoplastic cells was mostly trabecular with broad cords lined by sinusoids (Fig. 2). More solid areas were observed in some livers. A ductular growth pattern was noted in 1 liver. The neoplastic cells were basophilic with large vesicular nuclei which contained 1 or 2 distinct nucleoli (Fig. 3). Lipid droplets were present in some cells. In 2 livers large hyaline droplets were found in the cytoplasm (Fig. 4). Glycogen content was invariably decreased. Mallory bodies were not found. Mitotic figures were rarely observed. In some livers broad cords of fibrous stroma divided the neoplastic tissue. Areas with necrosis and haemorrhage were often noted.

Ultrastructurally, the tumor cells had rounded or elongated nuclei with irregular, large nucleoli. Euchromatin and some heterochromatin aggregations were observed along the nuclear envelope. Mitochondria were larger than normal and all cells were filled

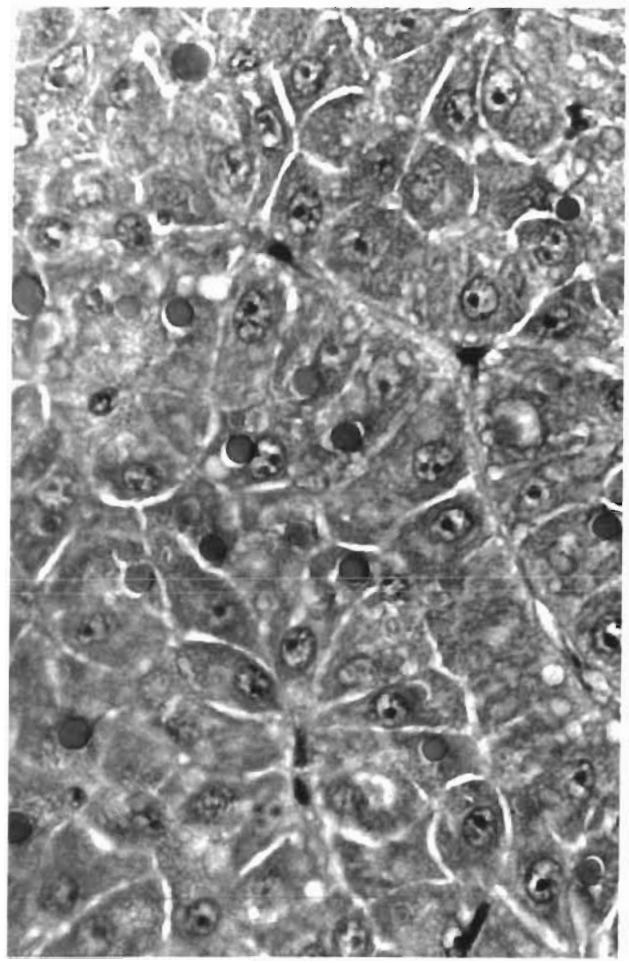

Fig. 4. *Salmo gairdneri*. Liver from individual with liver-cell carcinoma. Note hyaline droplets in some of the neoplastic cells. H-E, × 460

with rough endoplasmic reticulum which sometimes appeared slightly dilated and contained a homogeneous material (Fig. 5). Some cells had residual bodies or myelin figures in the cytoplasm (Fig. 5).

DISCUSSION

High prevalences of liver-cell carcinoma were recorded and at least 50 000 saltwater-reared rainbow trout were affected. It was demonstrated that only trout raised on the 1984 production of feed, containing cottonseed meal from Fodder Mill A, developed liver-cell carcinoma. Furthermore, aflatoxin B_1 was detected in batches of this feed suggesting that the outbreak of liver-cell carcinoma was associated with aflatoxin-contaminated feed. Aflatoxins were not found in examined livers because, most likely, the trout had been raised on uncontaminated feed for at least 6 mo before slaughter.

Prevalence of liver-cell carcinoma varied among marine trout farms. This may have been due to differences in management and in environmental factors at marine trout farms or at freshwater farms. Metabolic rate and feed intake are dependent upon water temperature and liver-cell carcinoma develops at a slow rate at low water temperatures (Wolf & Jackson 1963, Ashley 1973). Induction of liver-cell carcinoma in rainbow trout is also influenced by the genetic background of the fish (Wales 1970).

Observations on the morphology of liver-cell carcinoma coincided with previous descriptions (Scarpelli et al. 1963, Scarpelli 1967, Hendricks et al. 1984). Interestingly, neoplastic transformation of liver cells was observed in 1 macroscopically normal liver, suggesting that the actual prevalence could be higher than estimates based on the presence of macroscopical changes. Similar more conclusive observations have been reported before (Wood & Larson 1961).

The present investigation demonstrates that aflatoxin-contaminated feed from a single feed producer caused a substantial outbreak of liver-cell carcinoma among saltwater-reared rainbow trout in Denmark. The histological examination indicated that macroscopically normal livers could exhibit carcinoma.

Acknowledgement. This work was supported by the Agricultural and Veterinary Research Council, grant no. 3-3662.

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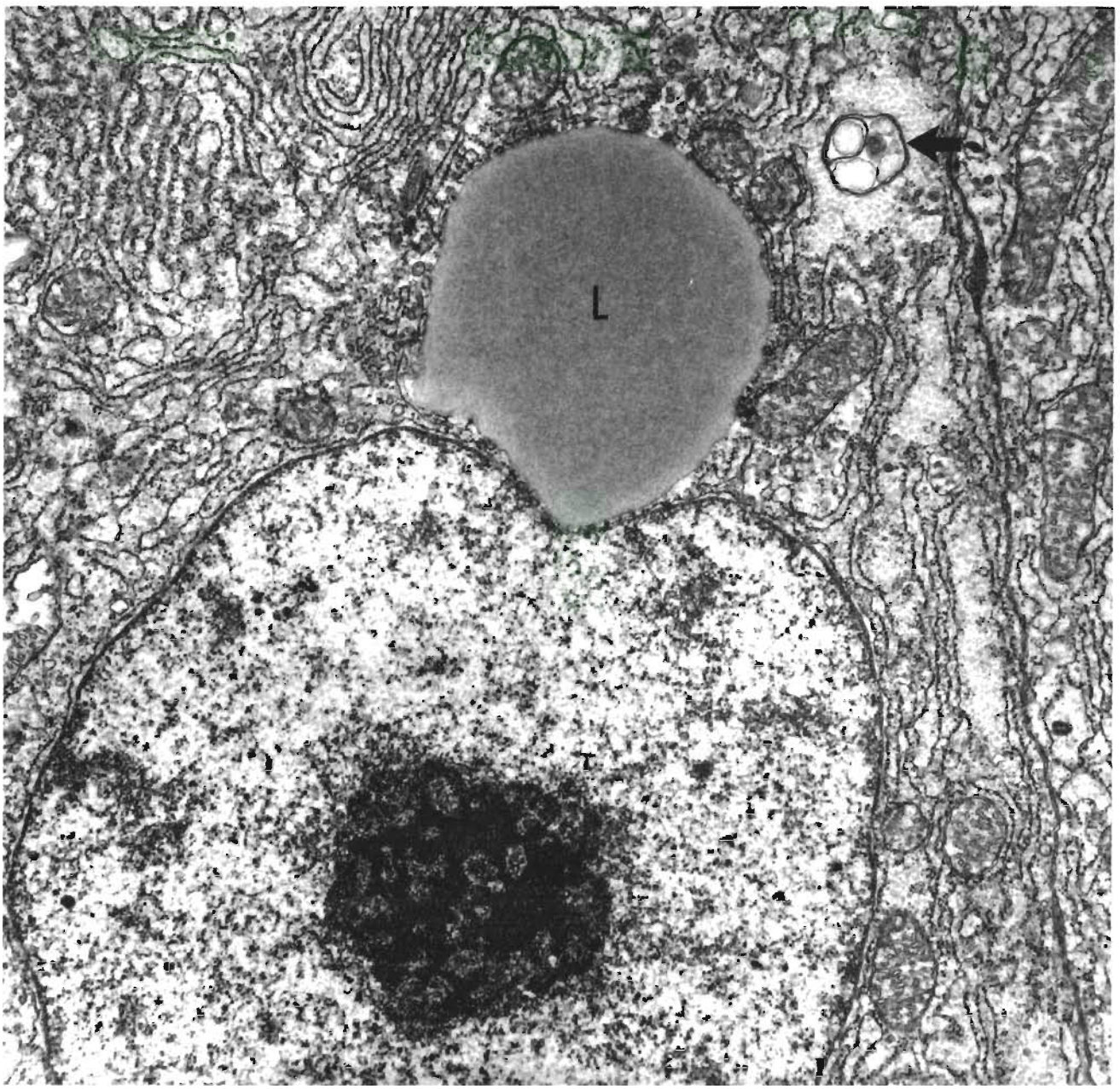

Fig. 5. *Salmo gairdneri.* Electron micrograph of neoplastic cell with abundant and slightly dilated rough endoplasmic reticulum. The cell contains a lipid droplet (L) and a myelin figure (Arrow). Uranyl acetate - lead citrate, × 18 500

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Responsible Subject Editor: Dr. O. Ljungberg; accepted for printing on July 21, 1986