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Viral Hemorrhagic Septicemia (VHS) in Pike (Esox lucius L.):

Clinical, macroscopic, histological and electron-microscopical findings; Direct visualization of the Egtved-virus.

Hämorrhagische Virusseptikämie (VHS) beim Hecht (Esox lucius L.): Klinische, makroskopische, histologische und elektronenmikroskopische Befunde; direkte Darstellung des Egtved-Virus.

by *W. Meier* and *K. Pfister**

Introduction

In a hatchery on the Hallwilersee – Switzerland – occured a loss of about 120000 pike fry (nearly 100%) during a 7-day period. The fry, originating from the Hallwilersee, had been kept in filtered lake water and were given a plankton diet for 3 weeks. The disease broke out shortly after an increase of the water temperature from 12 ° to 14 °–15 °C.

Macroscopic and virological examinations (Tab. 1) revealed the virus of viral hemorrhagic septicemia (Egtved virus), as the cause of the disease (*Meier* and *Vestergard Jorgensen,* 1979a).

Table I Reaction with Rabbit Anti F-1

	50% plaque neutr. titre	IFAT**
Pike-virus	800	+
* F-1	1500	+
* He	500	+
* 2375	300	+
Pike Fry Rhabdovirus	20	0
Rhabdovirus Carpio	20	0
Infectious Hematopoietic Necrosis Virus	20	0

* three serotypes of Egtved virus
** indirect fluorescent antibody technique

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In experimental trials with the isolated virus the disease was reproduced, so Koch's postulate was realized.

Viral hemorrhagic septicemia, (VHS) – hitherto only described in salmonids – is according to the Federal law of animal epidemic control a notifiable epidemic disease (1978). This viral disease causes the biggest losses in European trout farms. The losses in total trout production due to VHS are estimated as about 30% (*Ghittino* 1977; *Ghittino* et al., 1980). The present paper describes clinical, macroscopic, histopathologic and ultrastructural changes due to VHS in pike fry and demonstrates the Egtved virus in a direct electron microscopical visualization.

Material and Methods

Natural outbreak

The examined pike fry (2, 5–3, 5 cm) came from a hatchery on the Hallwilersee (Switzerland). Fish were kept in round basins at a water temperature of about 12 °C. The plankton diet and water were from the Hallwilersee. The outbreak of the disease occured in the early summer of 1978.

Fish of experimental reinfection

Two groups of 100 pike fry (2–3 cm) each, from the Moossee (Berne – Switzerland) were kept in 200 litre aquaria and supplied with tap water. The water temperature was about 12 ° to 15 °C, and a fresh plankton diet from Moossee was supplied daily. After a period of adaptation of 7 days, pike were infected experimentally with the isolated strain of the pike virus (*Meier* and *Vestergard Jorgensen,* 1979a). In one group the infection was carried out by 90 minutes exposure to 10^5 50% tissue culture infective doses ($TCID_{50}$) per ml of ambient water. The second group was infected by intraperitoneal inoculation of $10^4 TCID_{50}$ per fish.

Conventional histology

Fish for histological examination were fixed in Bouin's solution and in a potassium–phosphate buffered (pH 7,4) modified Karnovsky (2% Glutaraldehyde and 1,5% Paraformaldehyd), respectively. Tissue was embedded in paraffin or in Spurr low viscosity medium; 4–6 µ paraffin sections were stained with H + E; semithin (1–2 µ) Spurr sections were stained with toluidine-blue.

Electron microscopy

Tissue for electron microscopy was fixed in a potassium-phosphate buffered (pH 7,4) modified Karnovsky. Following postfixation in 1% OsO_4, tissues were dehydrated with acetone and embedded in Spurr's resin. The desired sites were selected from semithin sections. The ultrathin sections were contrasted with uranyl acetate and lead citrate and cut with an OmU_2 (Reichert) microtome. Observations and photographs were made with a Philips EM 300.

Results

1. Signs

Affected pike were apathetic and floated on their side at the surface of the water or remained at the bottom of the aquarium. The first signs were seen mostly 3–5 days after infection and nearly all affected pike died within 24 hours.

2. Gross lesions

Swelling with hemorrhages in the flanks and at the bases of the breast and tail fins were the most typical findings. Hemorrhages on the top of the head and on other regions of the body-surface were observed less often (Fig. 1). A slight bilateral exophthalmus was often seen. The gills were extremely pale. The abdomen was well-rounded and in most cases its cavity was filled by a clear, slightly yellowish, sometimes hemorrhagic fluid. The liver was pale, the intestines filled by a milky slime. Retroperitoneally, around the kidney and within the muscles, focal hemorrhages were evident (Fig. 2, 3).

3. Light microscopy

The hemorrhages on the top of the head could be located in the epidural area (Fig. 4) but rarely at other sites in the nervous system. Subretinal hemorrhages caused the exophthalmus. The retina was detached, often folded and compressed (Fig. 5). The pericardium often contained a serous, or sanguineous liquid (Fig. 6).

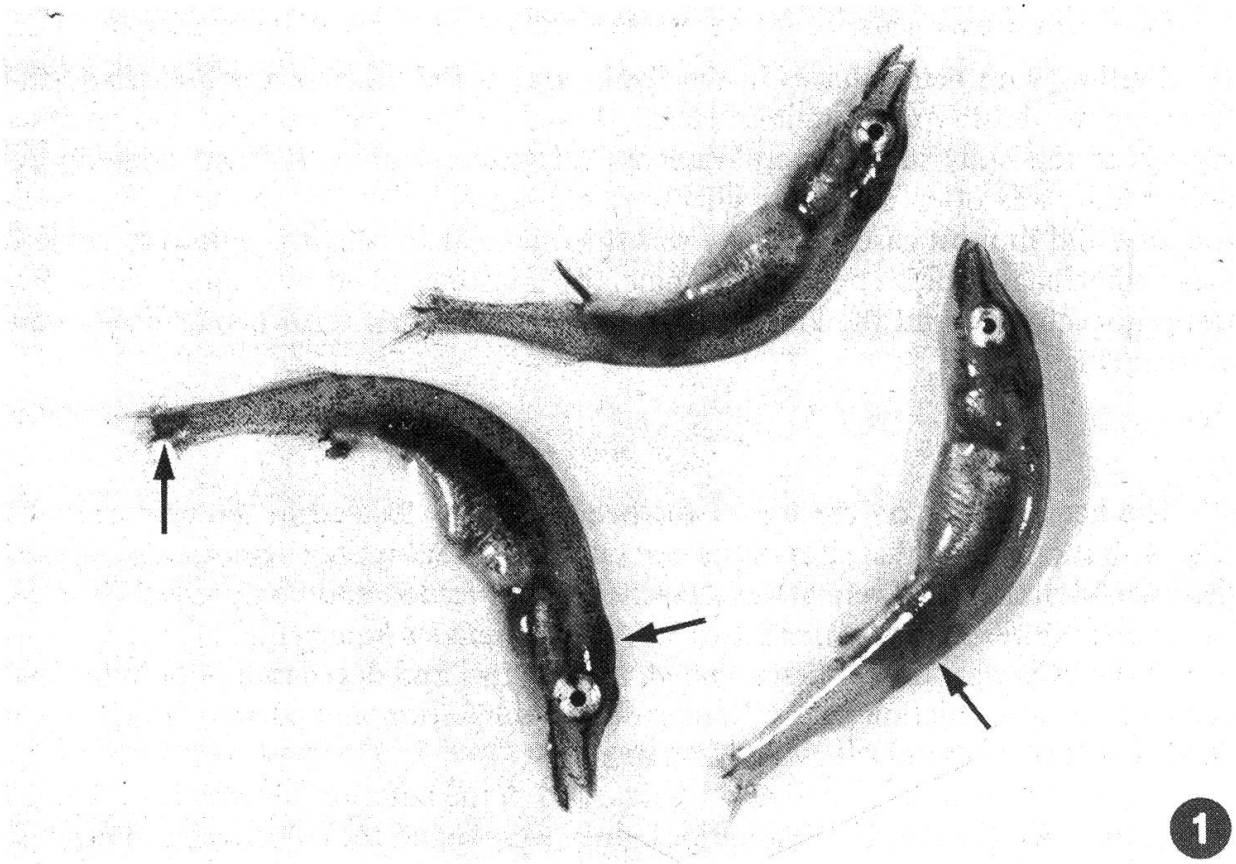
In the liver were found disseminated necrotic foci and degeneration of individual hepatocytes, also dilation of the sinuses due to congestion and edema. Similar focal necroses could be found in spleen and pancreas.

The most conspicuous lesions were detected in the anterior (hemopoietic) kidney and in the renal tubules. In the anterior kidney were found focal necrosis and degeneration of individual cells. Within necrotic foci remnants of capillaries with destroyed endothelium were evident, forming cavities partly filled with debris. A selective endothelial damage seemed to be responsible (Fig. 7). The tubules were irregularly altered, swollen, partly collapsed or compressed (Fig. 8). In swollen tubules the cell borders were not recognizable, epithelial cells were in some areas detached from the basal membrane. Many nuclei were swollen and vesicular or hyperchromatic and pyknotic. A characteristic finding in the cytoplasm was a marked granular pattern, reminiscent of inclusion bodies. Two types of granules, one with a coarse-grained and another with a fine-grained pattern, could be distinguished.

Often vacuolization of the cytoplasm was evident. The tubular lumen was sometimes filled with necrotic material or hyaline casts (Fig. 9, 10). The glomerula had a slightly swollen mesangium with deposition of serous material.

Electron microscopy

By ultrastructural investigation of the kidney the various degrees of cellular degeneration were more evident: Swollen, hyperchromatic and pyknotic nuclei were often together within the same tubule. Some nuclei had a prominent nucleolus. Homogenization or accumulation of chromatin at the inner nuclear membrane were noticed and a perinuclear space was often evident (Fig. 11, 12). The partly vacuolized cytoplasm varied in electron density and contained many mitochondria. The above described cytoplasmic granules proved either to be enormous amounts of sometimes swollen mitochondria, or conglomerates of swollen and degenerated mitochondria



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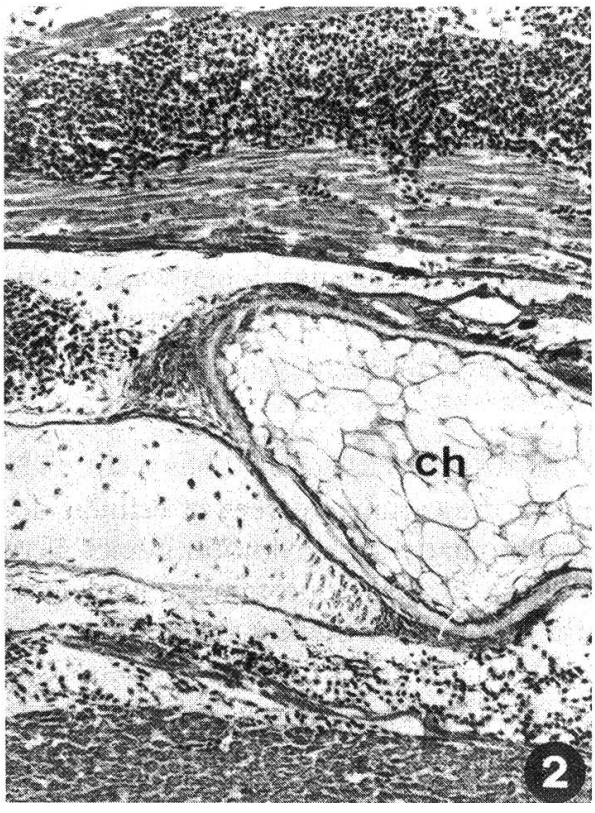

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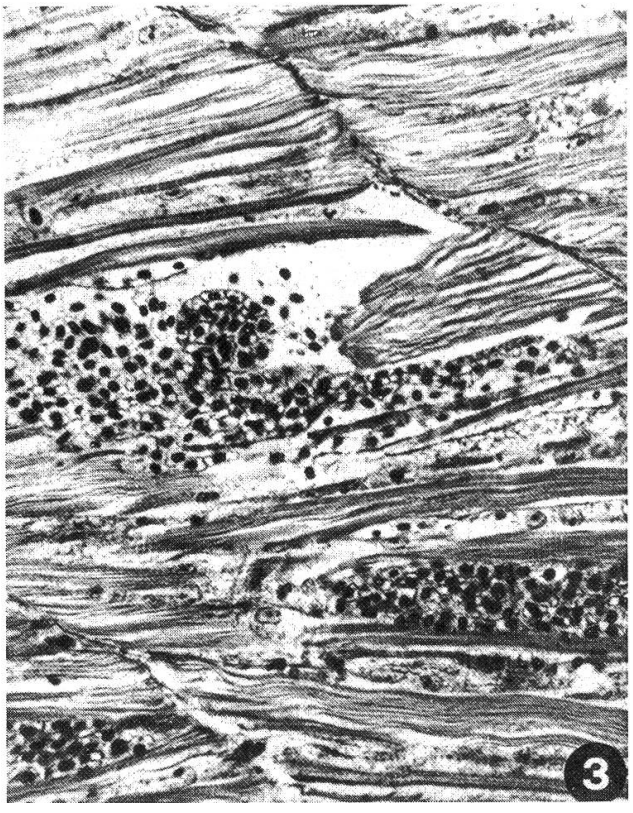

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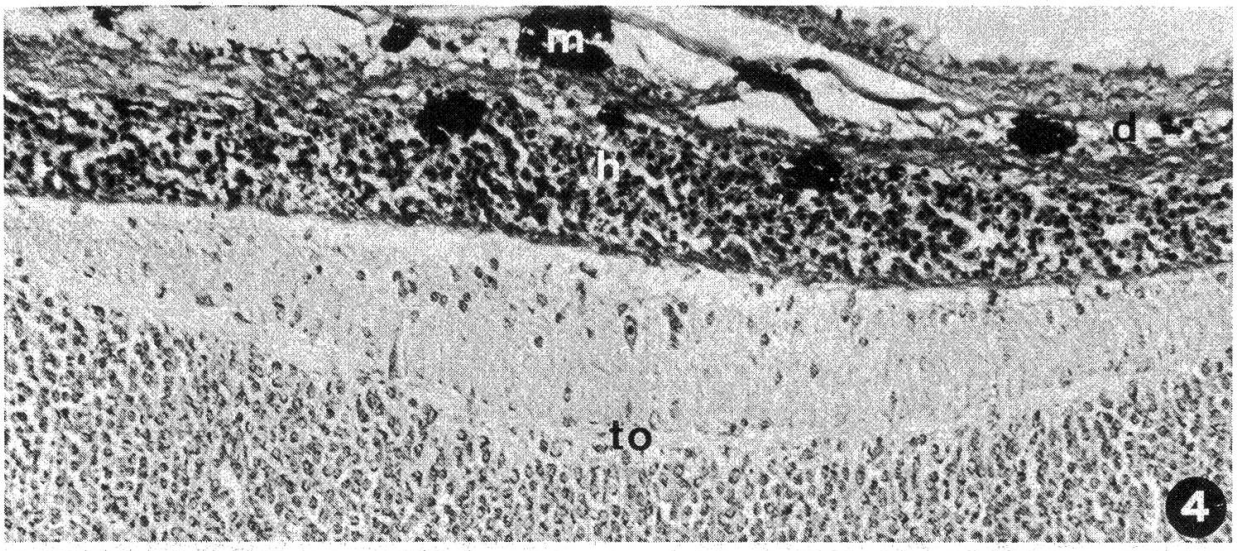
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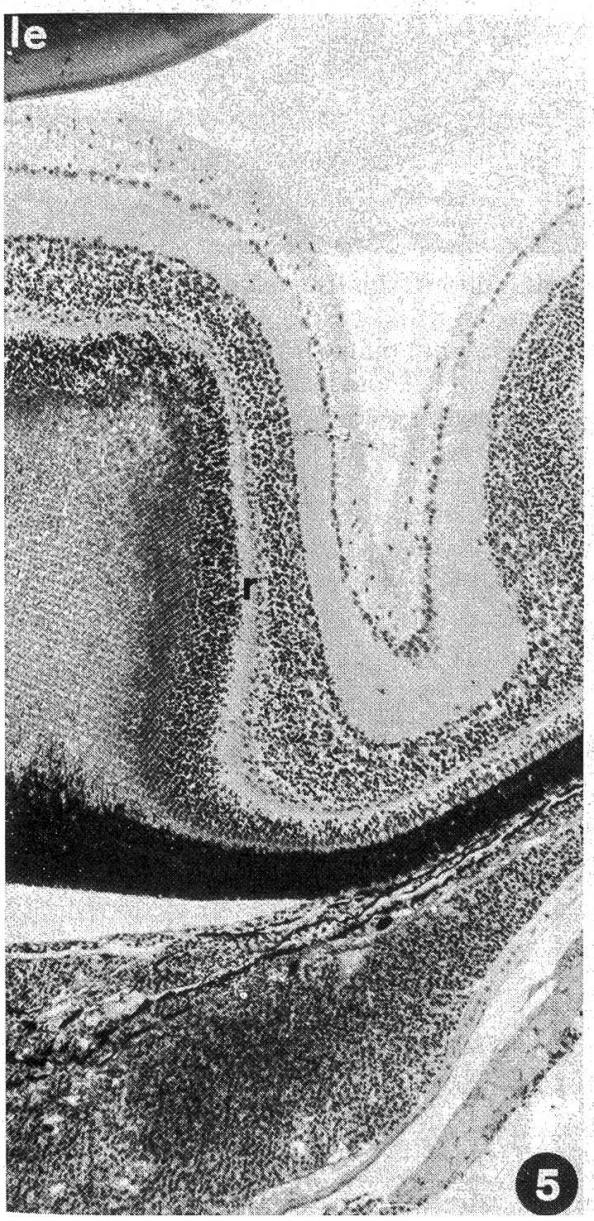
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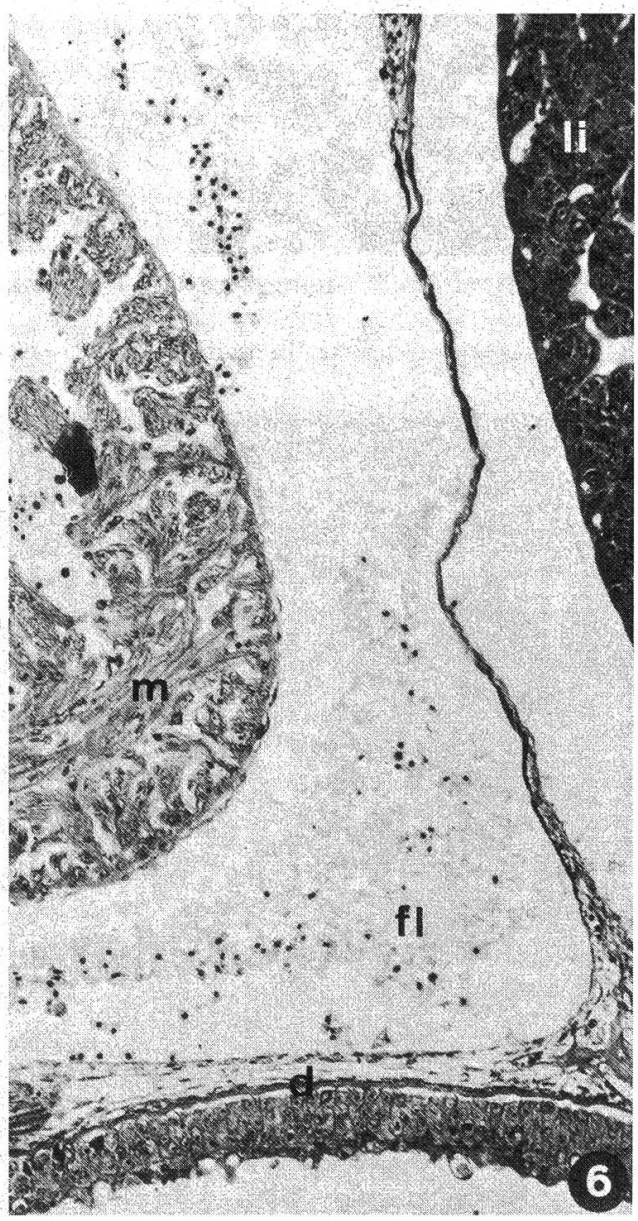
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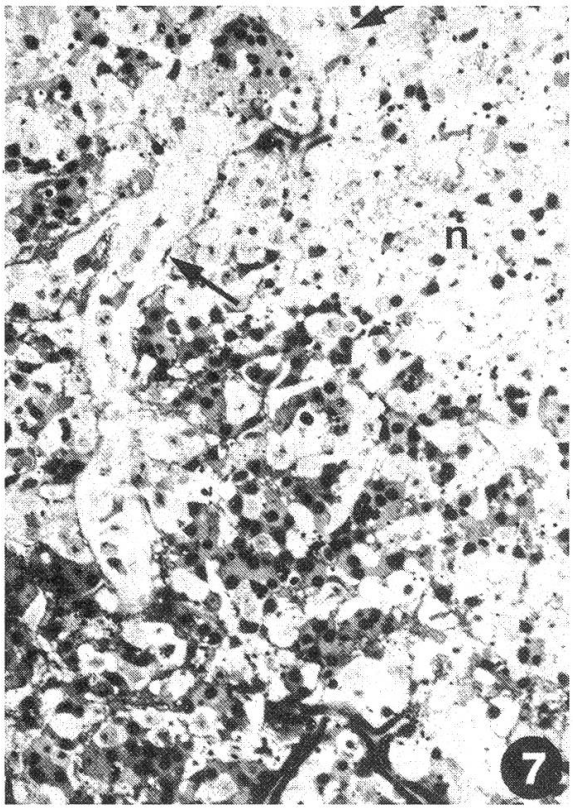
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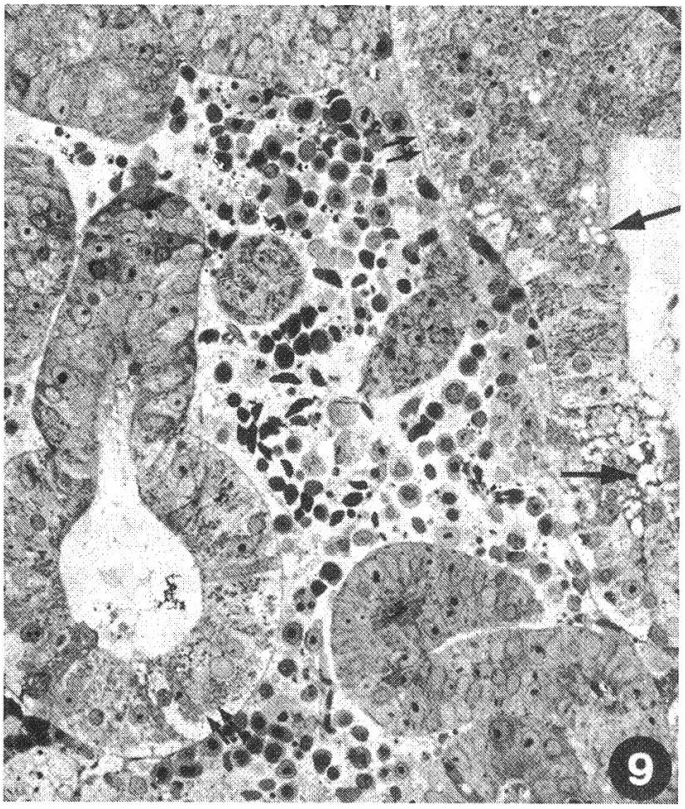
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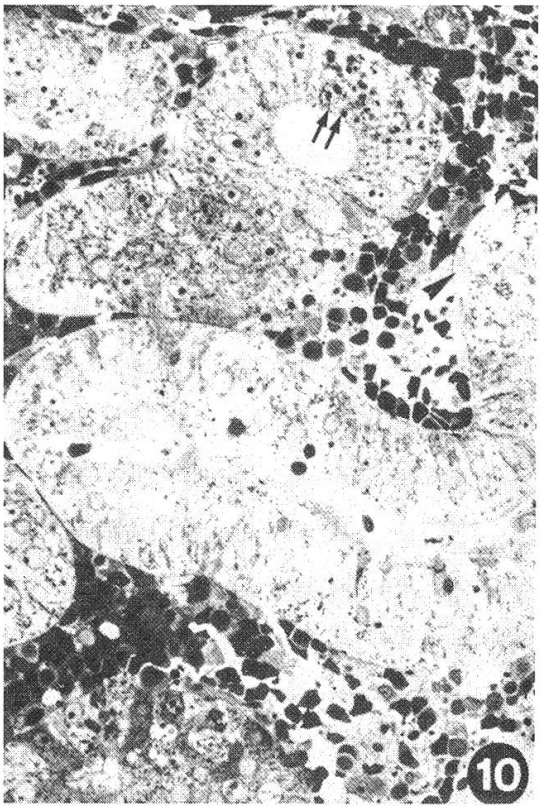
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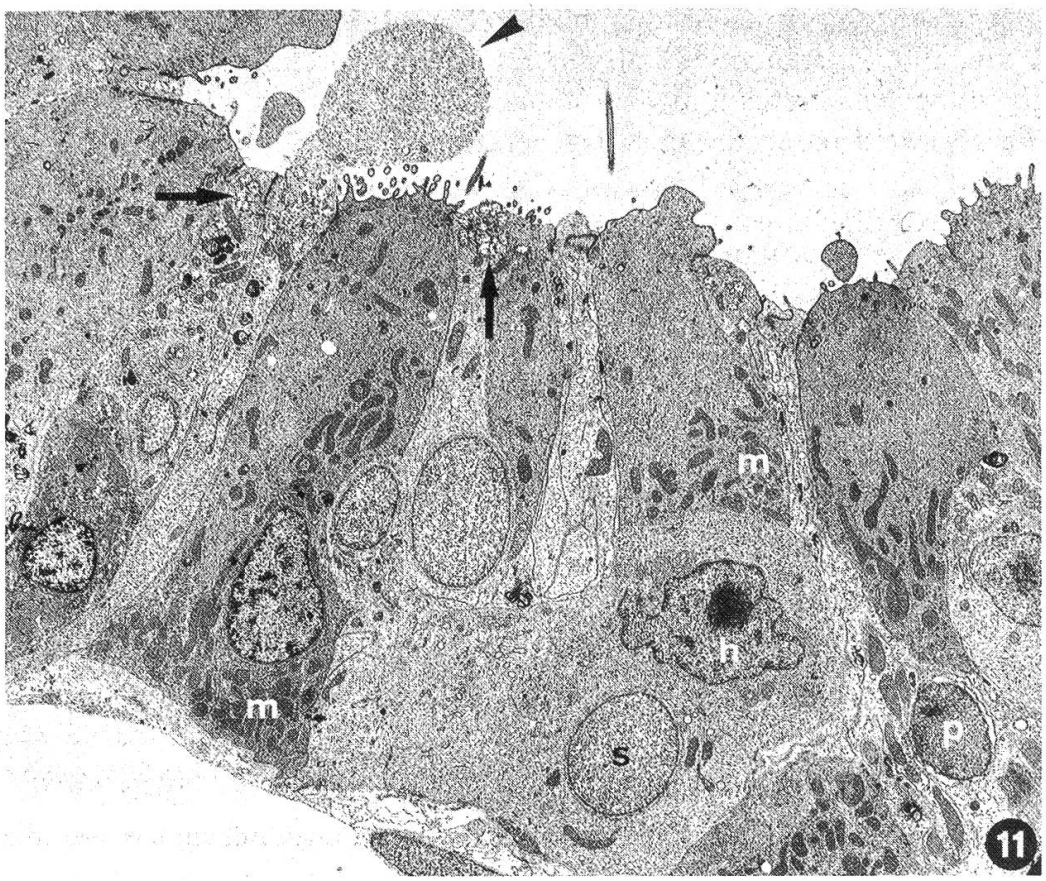
within phagolysosomes or as residual bodies (Fig. 11, 13, 14). The degranulated endoplasmic reticulum appeared very prominent, often encircling mitochondria. Tubules of rough endoplasmic reticulum were sometimes dilated (Fig. 12, 14). The number of microvilli appeared reduced. Some degenerated cells were ruptured into the tubular lumen (Fig. 11).

In some endothelial cells of the anterior kidney intracytoplasmic virus particles could be detected. The bullet-shaped structure of the virus and the size (200 × 70 nm), corresponded to the Egtved virus, the agent of VHS (Tab. 2).

Fig. 1 Pike with a dilated abdomen and widespread hemorrhages (↑)
2 × normal size
Fig. 2 Hemorrhages in the muscle of the tail; ch = chorda
HE; 130 ×
Fig. 3 Muscular hemorrhages
HE; 330 ×
Fig. 4 Epidural hemorrhages (h) in the tectum opticum area; to = tectum opticum; m = melanophores; d = dermis
HE; 160 ×
Fig. 5 Subretinal hemorrhages lead to detachment and folding of the retina (r); le = lens
HE; 130 ×
Fig. 6 Sero-sanguineous fluid (fl) within the pericardium; m = myocardium; li = liver; d = dermis
HE; 130 ×
Fig. 7 Necrotic area (n) within the anterior kidney; the endothelium of capillaries is destroyed (↑)
semithin section (ss); Tol. blue; 330 ×
Fig. 8 Highly damaged kidney with different stages of tubular changes. Partly swollen, and partly collapsed tubules are seen. Interstitial cells are normal in the fish kidney.
ss; Tol. blue; 130 ×
Fig. 9/10 Higher magnification of Fig. 8. Swollen epithelial cells are often vaculated (↑) and partly detached (▲). Nuclei are often vesicular or show hyperchromatosis. The cytoplasma has a typical granular pattern (↑↑).
ss; Tol. blue; 330 ×
Fig. 11 In affected tubular epithelial cells have undergone different stages of degeneration. Partly swollen nuclei (s) alternate with hyperchromatic (h) or pyknotic (p) ones. The cytoplasm is often vacuolated (↑) and mitochondria (m) are prominent. Some cells discharge their contents into the lumen (▲). The number of villi is partly reduced.
EM 3400 ×
Fig. 12 Nucleus of degenerated cell has an accumulation of chromatin (↑) at the inner nuclear membrane. The perinuclear space is dilated (↑↑). The endoplasmic reticulum (re), is degranulated and sometimes encircles mitochondria or other organelles.
EM 19200 ×
Fig. 13 Mitochondria have peculiar structure and size; within the same cell residual bodies (r) are often seen.
EM 9200 ×
Fig. 14 Highly damaged cell with a varying pattern of pathological organelles: m = altered mitochondria; f = myelin figures; ph = phagolysosomes packed with swollen mitochondria; dilated rough endoplasmic reticulum (↑)
EM 16200 ×
Fig. 15 Rhabdovirus within an endothelial cell (70 × 200 nm)
EM 143500 ×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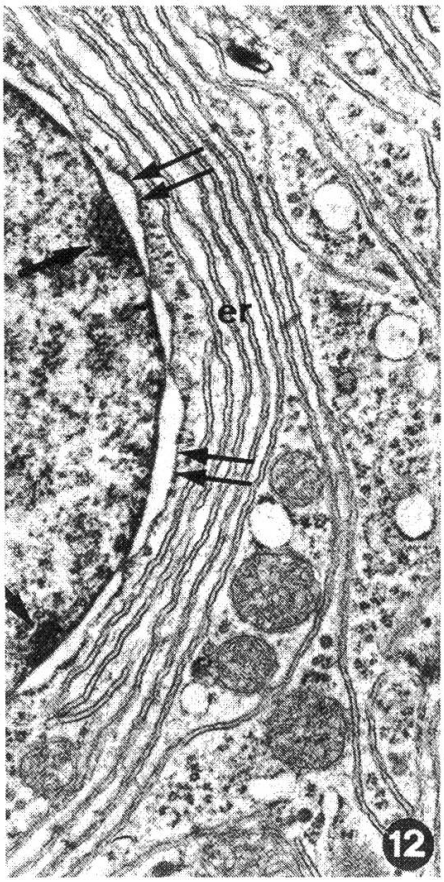
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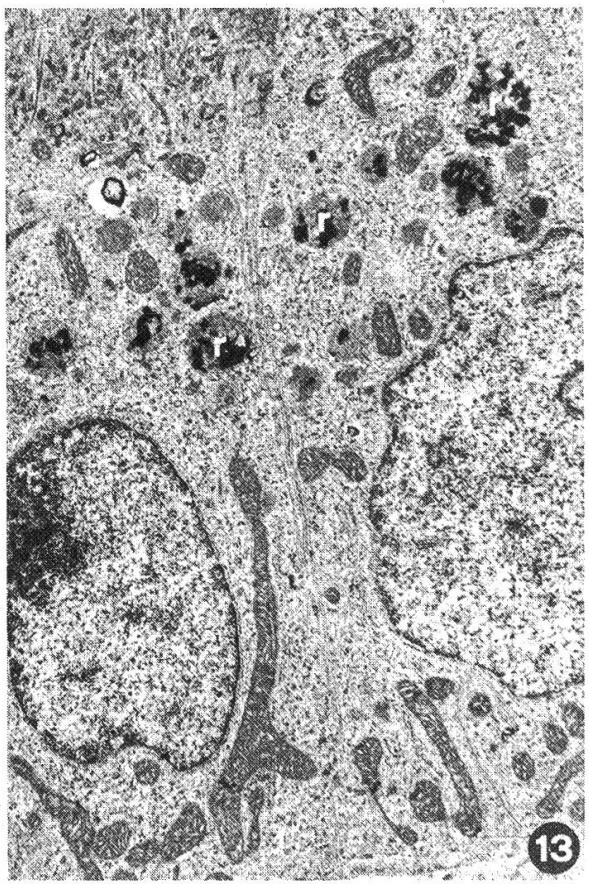
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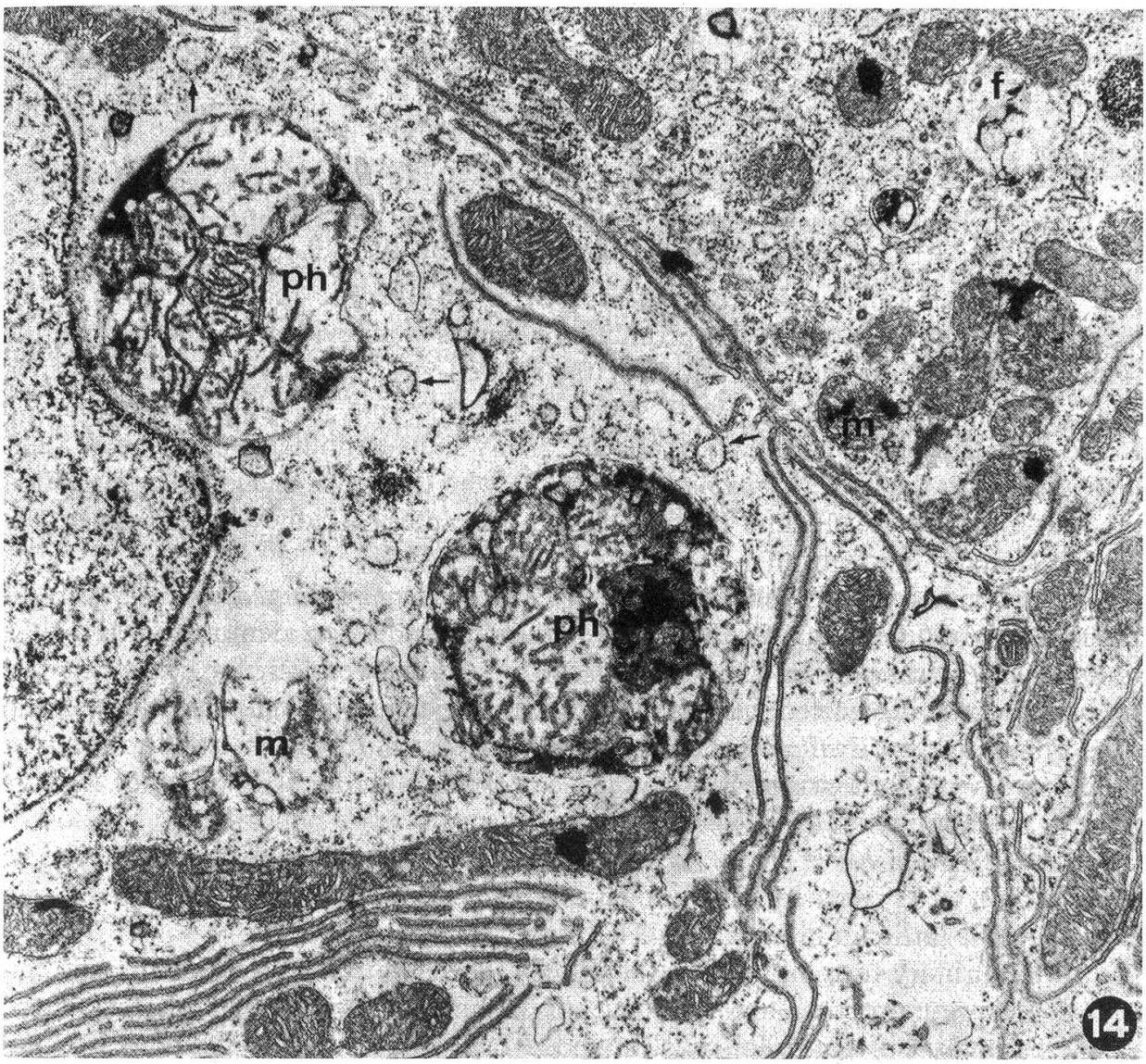
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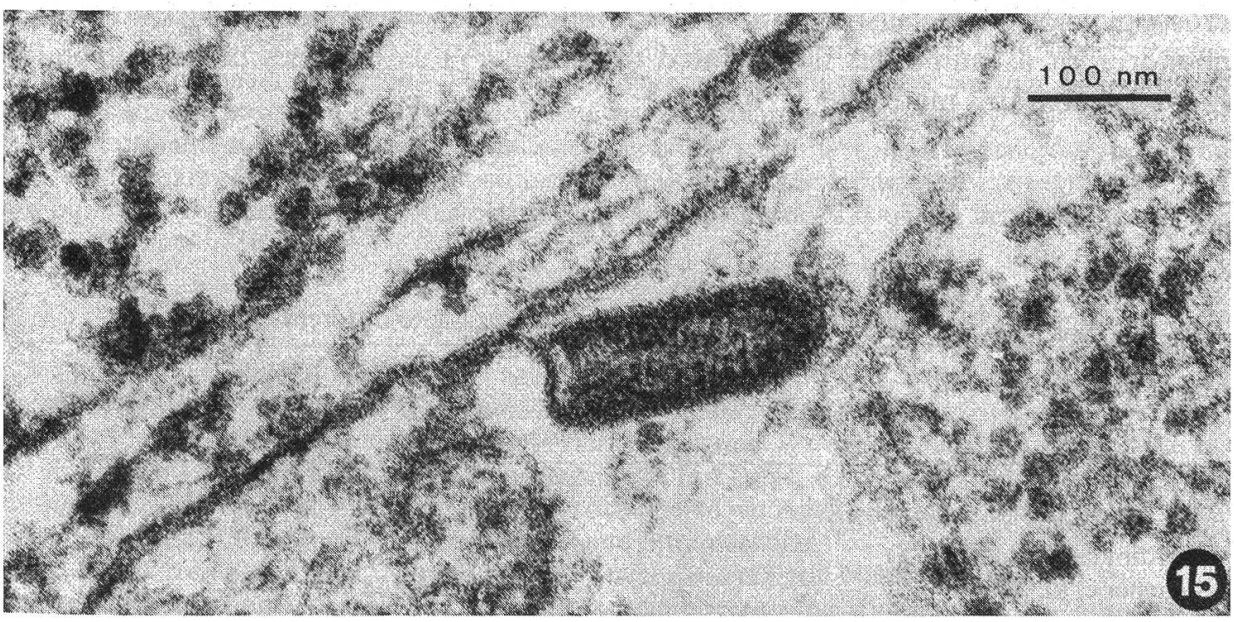
100 nm
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Discussion

The clinical course of the disease, the signs and the gross findings of examined pike fry coincided to a large extent with the acute form of viral hemorrhagic septicemia of young salmonids (*Ghittino,* 1967).

The very acute course of the disease with a mortality nearly 100% was impressive. Widespread hemorrhages in the skin and the muscles and exophthalmus were the most important signs. At post-mortem hemorrhages were observed retroperitoneally and around the kidney. By light microscopy small necrotic foci were observed in liver, spleen and pancreas. Very prominent changes were evident in the kidney, the target organ of virus-multiplication for the Egtved-virus (*Yasutake* and *Rasmussen,* 1968). In the anterior kidney were seen necrotic foci and in related capillaries we found degeneration of endothelial cells and many sinus-endothelial cells. These findings agree with those of *Hoffmann* et al. (1979), who described vacuolar degeneration of sinus endothelial cells and necrotic foci in the anterior kidney in experimentally VHS-infected rainbow trout.

Earlier virological investigations (*Meier* and *Vestergard Jorgensen,* 1979a, b) established the Egtved-virus as the cause of the disease. Direct demonstration of the bullet-shaped virus particle (70 × 200 nm) within capillary endothelial cells of the anterior kidney from affected pike and virological investigations confirmed the agent to be a Rhabdovirus (*Zwillenberg* et al., 1965; *Vestergard Jorgensen,* 1968). This first visualization of the Egtved-virus in pike corresponds with those of other authors done in rainbow trout (Tab. 2).

Recently *Amlacher* et al. (1980) accomplished the first direct electron microscopic visualization of Egtved-virus in affected rainbow trout; *Zwillenberg* et al. (1965) demonstrated it earlier in infected tissue cultures.

In the urinary forming part of the kidney, degenerative changes of tubular epithelial cells were predominant. By conventional histology a marked granular pattern of the cytoplasm of epithelial cells reminded one of inclusion bodies. Electron microscopy showed the suspected inclusion bodies either as an accumulation of mitochondria, or as numerous degenerated organelles. Virus particles could not be demonstrated in this material.

Table II Egtved-virus: morphological characteristics according to different authors

	length: nm	width: nm	proof in cell culture	direct
Zwillenberg et al. (1965)	180	60–70	x	
de Kinkelin and Scherrer (1970)	240	75	x	
Olberding and Frost (1975)	165	65	x	
Amlacher et al. (1980)	166–216	55–68		x

All these changes are of nonspecific degenerative character and do not allow one to draw conclusions on the etiology. Our cases had a severe tubulonephrosis unlike the findings of *Hoffmann* et al. (1979), who pointed out that induced vascular lesions and disturbance in thrombopoesis are of pathogenetic importance. These factors could – varying with the dose of infection and the virulence of the virus – be secondarily responsible for the tubulonephrosis.

In the differential diagnosis, Pike Fry Rhabdovirus Disease, one of the most important hitherto known viral diseases of pike fry must be considered. According to *Bootsma* (1976) this disease affects pike of the same age, causes the same signs and indistinguishable macroscopic and microscopic lesions. It is therefore absolutely necessary – if these signs are seen – to make a diagnosis only after a virological examination. Pike Fry Rhabdovirus Disease has not yet been demonstrated in Switzerland.

This first outbreak of VHS in pike is, both from the economical and from the epidemiological point of view, of importance:

1. VHS may occur in pike as well as in salmonids and causes severe losses in pike fry.
2. It may be that pike contribute to a spread of the Egtved-virus. In Switzerland this is of importance in the control of notifiable epidemic fish disease (Eidg. Tierseuchengesetzgebung, 1978). The virus can be transferred directly by affected animals, or older animals may act as carriers of a latent infection and thus excrete virus (*Meier,* 1980, unpublished). In countries like Switzerland, where several millions of pike fry and several tons of pike are released in all appropriate waters, pike can contribute to a latent VHS-infection of waters. In breeding stations, moreover, pike could be a direct source of infection for salmonids.

Summary

A spontaneous outbreak of viral hemorrhagic septicemia (VHS) in pike fry from a Swiss fish-breeding station is described. The first isolation and virological characterization of the agent was published earlier (*Meier* and *Vestergard Jorgensen,* 1979). The mortality in the affected pike population was nearly 100%. The main signs were exophthalmus and hemorrhages in the flanks, on the skull and at the bases of the fins. Pathological-anatomical findings were: anemia, hemopericardium, ascites and extensive retroperitoneal and intramuscular hemorrhages. By light microscopy, the most prominent findings were disseminated necrotic foci in liver, spleen, pancreas and in the hemopoietic part of the kidney. In the urinary-forming part of the kidney a severe tubulonephrosis was observed. By direct electron microscopy, Egtved-virus particles could be demonstrated in the anterior kidney.

Zusammenfassung

Ein Spontanausbruch von Hämorrhagischer Virusseptikämie (VHS) bei Hechtbrütlingen in einer schweizerischen Fischzucht wird beschrieben. Die Erstisolation und virologische Charakterisierung des Erregers wurden bereits früher publiziert (*Meier* und *Vestergard Jorgensen,* 1979a). Bei einer Mortalität von nahezu 100% fielen Blutungen u.a. in Flanke, Kopf und Flossenansätzen sowie Exophthalmus auf. Pathologisch-anatomisch beobachteten wir Anämie, Hämopericard, Ascites, sowie ausgedehnte Hämorrhagien retroperitoneal und intramuskulär.

Lichtmikroskopisch imponierten vor allem fokal-disseminierte Nekrosen in Leber, Milz, Pankreas sowie im hämopoietischen Teil der Niere. Im harnbereitenden Teil bestand eine ausgeprägte

Tubulonephrose. Im Kopfnierenbereich konnten direkt elektronenoptisch geschossförmige Egtved-virus-Partikel dargestellt werden.

Résumé

Cet article rapporte le cas d'une apparition spontanée dans une pisciculture suisse de septicémie hémorragique virale chez des brochets alvins. Une publication concernant l'isolation primaire du microbe, de même que l'étude virologique de ses caractères a déjà paru (*Meier* et *Vestergard Jorgensen,* 1979a). La mortalité s'élevait à presque 100%. Le plus frappant sont des hémorragies, avant tout localisées au niveau des flancs, de la tête, à la base des nageoires, et de l'exophtalmie.

Du point de vue anatomopathologique, l'on remarqua de l'anémie, un hémopéricarde, de l'ascite de même que des hémorragies rétropéritonéales et intramusculaires étendues.

Au microscope optique on distingue particulièrement des foyers de nécrose dans le foie, la rate, le pancréas ainsi que dans la partie hémopoiétique des reins. Dans la partie sécrétrice des reins on constata une tubulonephrose avancée. Dans la région de la tête des reins, la microscopie électronique a permis d'observer directement des particules en forme de projectile de virus d'Egtved.

Riassunto

Si descrive un episodio spontaneo di setticemia virale emorragica (VHS) in avanotti di luccio di un allevamento svizzero. L'isolamento e la caratterizzazione virologica dell'agente infettivo sono stati già pubblicati (*Meier* e *Vestergard Jorgensen,* 1979a). La mortalità è stata di circa il 100%; clinicamente si sono osservate emorragie, soprattutto nella regione dei fianchi, della testa e dell'attacco delle pinne, ed esoftalmo. Da un punto di vista anatomopatologico sono state osservate anemia, emopericardio, ascite, estese emorragie retroperitoneali e intramuscolari.

Le indagini istopatologiche hanno messo in evidenza soprattutto necrosi focali disseminate nel fegato, nella milza, nel pancreas e nella porzione emopoietica dei reni. Nella porzione urinoproduttrice dei reni si è reperita una accentuata tubulonefrosi. Le indagini ultrastrutturali hanno permesso di osservare particelle di virus di Egtved a forma di proiettile nella regione della testa dei reni.

Acknowledgements

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REFERATE

Beobachtungen über die Bakterienflora von Abszessen bei Schweinen, von J.E.T. Jones.
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Abszesse von Schweine-Schlachtkörpern, Alter 5–6 Monate, und Euterabszesse von Sauen wurden aus Schlachthöfen besorgt. Eiterproben von jedem Abszess wurden auf je zwei 6% Pferdeblut-Agrarplatten verimpft und 4 Tage bei 37 °C bebrütet, je eine aerob und anaerob.

Im ganzen resultierten 213 Isolate aus 93 von 100 Schlachtkörper-Abszessen und 101 Isolate aus 48 von 50 Gesäugeabszessen. Die Zusammensetzung der Isolate aus beiden Gruppen war ungefähr gleich. Die vorherrschenden Keime waren Corynebacterium pyogenes, beta-hämolytische Streptokokken, Clostriden, Bacteroides spp. und unklassifizierte anaerobe, grampositive Kokken. Die Resultate sind in den Tabellen I–III zusammengestellt.

Tab. I Identifikation von 213 Bakterienisolaten aus 93* von 100 Schlachtkörper-Abszessen in 5–6 Monate alten Schweinen.

Aerob	Zahl Isolate	Anaerob	Zahl Isolate
Corynebacterium pyogenes	71	Clostridium spp.	44
Streptococcus spp.	22	Gram-positive Kokken	16
Pasteurella multocida	11	Bacteroides spp.	11
Escherichia coli	7		
Staphylococcus aureus	6		
Staphylococcus epidermidis	5		
Verschiedene +	9		
Nicht-identifiziert	11		
Total	142		71

* Aus 7 Abszessen wurden keine Bakterien isoliert

\+ Nicht-identifizierte Arten von: Aeromonas (3), Chromobacterium (2), Proteus (2), Acinetobacter (1) und Haemophilus (1)