TROUT MORTALITIES AS A RESULT OF *STREPTOCOCCUS* INFECTION*

J. BOOMKER([1]), G. D. IMES Jr([2]), C. M. CAMERON([3]), T. W. NAUDE([4]) and H. J. SCHOONBEE([5])

ABSTRACT

BOOMKER, J., IMES, G. D. Jr, CAMERON, C. M. NAUDE, T. W. & SCHOONBEE, H. J., 1979. Trout mortalities as a result of *Streptococcus* infection. *Onderstepoort Journal of Veterinary Research*, 46, 71–77 (1979).

Excessive mortalities were experienced in the bigger rainbow trout (*Salmo gairdneri*) at an efficiently managed trout farm. All tests for known toxins in the feed and water proved to be negative. A faecal *Streptococcus* which belongs to the Lancefield group D but which could not be identified as belonging to any of the recognized species within this group, was isolated from the spleens, livers and kidneys of affected fish. Pathogenicity studies with this organism proved it to be highly fatal to trout but not to Mozambique bream (*Sarotherodon mossambicus*), banded bream (*Tilapia sparrmanii*), carp (*Cyprinus carpio*) or largemouth bass (*Micropterus salmoides*). The isolation and biochemical characteristics of the organism are described. The symptoms, gross- and histopathology of this disease are described and discussed. The disease resembles a haemorrhagic septicaemia and appears to be associated with intensification and conditions of stress.

Résumé

MORTALITÉ CHEZ LA TRUITE DUE A UNE INFECTION A STREPTOCOCCUS

On a constaté une mortalité excessive chez la grande truite arc-en-ciel (Salmo gairdneri) *dans un élevage pourtant bien organisé. Tous les tests pour toxines connues dans l'eau et la nourriture se sont avérés négatifs. Un* Streptococcus *fécal, appartenant au group Lancefield D, mais qu'on n'a pu rattacher à aucune des espèces connues de ce groupe, a été isolé des rates, des foies et des reins du poissons affectés. Des recherches de pathogénicité entreprises avec cet organisme ont montré qu'il est très souvent mortel pour la truite, mais pas pour la brème du Mozambique* (Sarotherodon mossambicus), *ni pour la brème rayée* (Tilapia sparrmanii), *la carpe* (Cyprinus carpio) *ou la perche à large gueule* (Micropterus salmoides). *On décrit le procédé d'isolement et les caractéristiques biochimiques de cet organisme. On décrit aussi et l'on discute les symptômes, la pathologie macroscopique et l'histopathologie de cette maladie. Elle ressemble à une hémorrhagie septicémique et semble être associée à l'intensification et à des conditions de stress.*

INTRODUCTION

Excessive mortalities caused by a condition aptly described as "pop-eye" were experienced in October 1974 in the bigger rainbow trout (*Salmo gairdneri*) on an extensive and efficiently managed farm in the Magaliesburg district of the Transvaal (altitude 1 472 m). On the affected farm, apparently healthy fish were either found dead or they died after swimming around erratically for some time. Dark pigmentation of the skin and "pop-eye", with one or both eyes showing moderate to severe exophthalmos, were frequently encountered in the affected fish. Haemorrhages in the eye chamber or behind the eyes were often seen and the loss of one or both eyes through rupture was a frequent occurrence. Isolated cases of "pop-eye" had occurred previously, but mortalities increased after the fish had been subjected to stress as a result of temporary overcrowding while new ponds were being constructed.

The farm is situated in a dolomitic area and the water is supplied by a group of very strong fountains situated about 100 m above the trout ponds. The water is of high purity and, since the temperature is consistently 19 ($\pm$1) °C throughout the year, temperature changes could not be incriminated as a cause of mortality. Apart from a few small specimens of an indigenous *Barbus* species in a pond in between the source of the water supply and the trout ponds, the trout were the first fish to be exposed to this water. The existing ponds were constructed at 2 levels. After the water had been utilized in the ponds at the upper level, it was mixed with fresh water and re-used at the lower level. The excessive mortalities were experienced initially in one of the upper ponds, then spread to the whole of the lower level and finally back to some ponds in the upper level. Certain ponds, however, remained unaffected.

Early attempts to relate this condition to feed batches or different brands of trout pellets were unsuccessful. A change of feed from pellets to fresh liver and meat had no apparent effect, nor could the mortalities be related to oxygen, ammonia or nitrate content, as determined by standard procedures. Subsequently, similar mortalities occurred in rainbow trout on a farm lower down the same stream and also on another farm in the same region, where a different water source was utilized.

A *Streptococcus* sp. was isolated from the first fish submitted for examination, and the results of the preliminary investigation were reported by Naudé (1975). *Streptococcal* infection in rainbow trout was subsequently also described by Roode (1977).

The objective of this investigation was to determine the role played by the *Streptococcus* in the aetiology of the disease. Bacteriological, pathological, toxicological and pathogenicity studies were undertaken for this purpose.

* Presented in part at the Biennial Congress of the South African Veterinary Association, Durban, 1975
The views expressed herein are those of the authors and do not necessarily reflect the views of the U.S. Air Force or Department of Defence

([1]) Department of Parasitiology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, Onderstepoort 0110. Formerly Dept. of Pathology, Veterinary Research Institute, Onderstepoort 0110

([2]) Lt.-Col., U.S.A.F., VC. Chief, Zoonotic Pathology Section, Armed Forces Institute of Pathology, Washington, DC, U.S.A.

([3]) Department of Bacteriology, Veterinary Research Institute, Onderstepoort 0110

([4]) Department of Toxicology, Veterinary Research Institute, Onderstepoort 0110

([5]) Department of Zoology, Rand Afrikaans University, P.O. Box 524, Johannesburg 2000

MATERIALS AND METHODS

Investigation of natural outbreaks

Fish that had recently died were collected on the premises and specimens for bacteriological examination were removed aseptically. Cultures were prepared from the liver, spleen and kidneys on bovine blood tryptose agar (Difco) (BTA) plates which were incubated at 37 °C for 18–24 h. To prepare an injectable suspension for pathogenicity studies, 18-hour-old cultures were prepared on BTA plates. The growth was washed off with tryptone water and the suspension nephelometrically adjusted to contain approximately 10^9 bacteria/mℓ.

An antibiogram was prepared, using standard disc techniques.

Blood and organ smears of infected fish were also prepared according to standard techniques, and stained with Giemsa's stain. Tissues were collected and fixed in 10% neutral buffered formalin. After being embedded in paraffin wax, sections were cut at 5 μm thickness and stained with haematoxylin and eosin (HE) and with Wohlbach's modification of Giemsa's staining method (Thompson, 1970).

The different batches and brands of trout pellets were collected and tested for aflatoxin.

Water specimens were collected and tested for organo-phosphorus and chlorinated hydrocarbon insecticides as well as aflatoxin, according to standard methods.

Pathogenicity studies

Experiment 1. Nine apparently healthy rainbow trout about 15–20 cm long collected from the unaffected ponds in the upper section of the affected farm were transported from the farm to the laboratory. They were kept in a large glass aquarium through which water at 20 °C was continuously circulating. After a period of adaptation, 2 fish were injected intraperitoneally and 2 intramuscularly with 1 mℓ of a bacterial suspension containing 10^9 organisms. The injected fish were returned to the aquarium with the remaining 5 controls.

Experiment 2. Experiment 1 was repeated with 23 trout originating from the Fisheries Institute, Lydenburg. The fish were kept together in a large glass aquarium at 20 °C and were allowed a 15-day adaptation period. At the beginning of the experiment, 4 fish were sacrificed for bacterial examination to exclude the presence of *Streptococcus* in the various organs. Four fish were injected intramuscularly and 4 intraperitoneally with 10^9 organisms as above. The remaining 11 fish served as in-contact controls.

Experiment 3. The above experiments were repeated with 18 rainbow trout 10–15 cm long, also originating from the Fisheries Institute, Lydenburg. In this case, however, the fish were divided into 3 groups of 6 each, and the groups kept separately in 3 smaller glass aquaria. One group was injected intraperitoneally, a 2nd group intramuscularly, while the 3rd group was not injected. The injected fish received the same dose of bacteria as those in the first 2 experiments.

Experiment 4. The experiment was done with Mozambique bream (*Sarotherodon mossambicus*), banded bream (*Tilapia sparrmanii*), carp (*Cyprinus carpio*) and largemouth bass (*Micropterus salmoides*) at different temperatures. All these fish were about 10–15 cm long.

In this experiment the fish were divided into 3 subgroups, each consisting of 6 fish, and kept at 20–23 °C. A similar group of 18 fish of the same species was kept at 30–33 °C. The 6 subgroups were kept separately in glass aquaria. One subgroup of each main group was injected intraperitoneally, the 2nd intramuscularly, while the 3rd subgroup was not injected, but kept as a control. The subgroups received the same number of bacteria as the trout in the previous experiments.

Bacteriological specimens were taken, blood and organ smears prepared and tissues collected for histopathology from all fish that died during the course of the experiments.

RESULTS

In Experiments 1 and 2 all the injected fish died and the condition spread to the in-contact controls. In Experiment 3, the 12 injected fish died but the controls remained alive and unaffected. In Experiment 4 only 1 of the Mozambique bream which had been injected intra-peritoneally died acutely. None of the other species of fish, nor the remaining Mozambique bream showed any symptoms and were still alive and actively feeding after 30 days.

Clinical signs

The trout that were experimentally infected became lethargic 1–3 days after the injection, swam near the surface and refused to eat. Exophthalmos, affecting one or both eyes, developed rapidly (Fig. 1). Haemorrhages developed in and behind the eyes and the lenses became cloudy. At this stage the fish with both eyes affected turned almost completely black, while those with only one eye affected developed a patchy dark pigmentation on the dorsal skin. Occasionally a fish with a distended abdomen and a slightly protruding rectum was seen. Affected fish tended to keep away from the healthy ones and swam about erratically. Death usually occured 2–4 days after injection, but in the first 2 experiments some deaths occurred after 24 h. In the first 2 experiments, where the controls were in the same tank as the infected ones, the controls started dying after 5 days. In the 3rd experiment, where the groups were kept separately, the controls did not develop any symptoms.

The bacterium was isolated from the spleens, livers and kidneys of all the trout and of the one Mozambique bream that died during the course of the experiments.

Bacteriology

In most cases where material was examined, cultures were virtually pure and the kidneys usually yielded the largest number of colonies on primary isolation.

Colonies on BTA plates were approximately 1–2 mm in diameter, dull grey in colour and surrounded by a narrow zone of weak beta-haemolysis. Gram-stained smears revealed small single Gram-positive coccobacilli which sometimes occurred in pairs or short chains.

The organism was non-motile and grew at 45 °C but not at 10 °C or 50 °C. It grew readily on Mac-Conkey agar and produced neither oxidase nor catalase. It did not grow in 1% methylene blue milk, and litmus milk was unchanged after 7 days incubation at 37 °C. Esculin and sodium hippurate were both hydrolized. Gelatin was not liquefied. Acid, but no gas was produced from glucose (final pH 4,0), starch, maltose, trehalose, lactose, salicin, galactose (late) and dextrin (late), nor was acid produced in sorbitol, arabinose, sucrose, glycerol, mannitol, inulin, raffinose, dulcite, inosite or xylose.
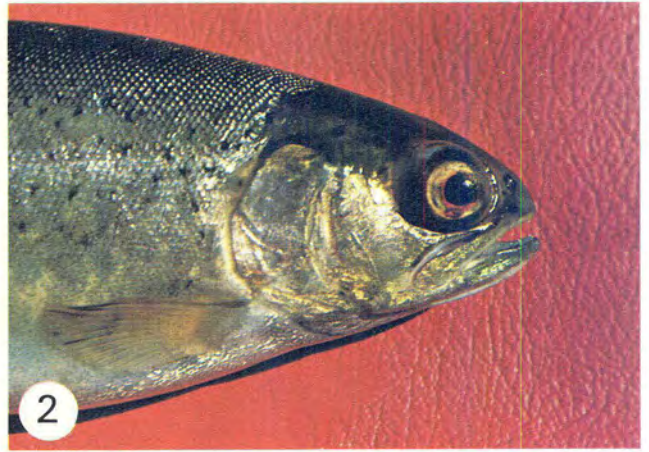
FIG. 1 Rainbow trout, dorsal view, showing exophthalmos, haemorrhages in eye and dark dorsal discoloration
FIG. 2 Haemorrhage in anterior chamber of eye of rainbow trout
FIG. 3 Marked splenomegaly and diffuse petechiae, sub-peritoneally
FIG. 4 Haemorrhage and petechiae in a slightly discoloured liver

On the basis of the above characteristics, the organism was identified as a faecal *Streptococcus* species and serologically it was found to belong to Lancefield group D. Its biochemical characteristics, however, were such that it could not be identified as belonging to any one of the recognized species within this group (Buchanan & Gibbons, 1974).

The antibiogram indicated that the organism was sensitive to tetracycline and the addition of 3 500 ppm tetracycline to the feed reduced the mortalities on the farm where the original outbreak occurred to the normal percentage of about 5%. However, the problem recurred as soon as tetracycline medication was stopped.

Toxicology

All the specimens of the different batches and brands of trout pellets were negative for aflatoxin. The water and fish which were collected at the farm were negative for organo-phosphorus and chlorinated hydrocarbon compounds.

Pathology

Cross Pathology—Natural cases

Fish that died acutely showed little gross pathological changes. The most pronounced lesions were mild peri- and intraocular haemorrhages with exophthalmos (Fig. 1 & 2). The internal organs showed mild changes, including an enlarged liver and spleen (Fig. 3). Petechial haemorrhages were consistently seen in the peritoneum and subperitoneal muscles (Fig. 3 & 4).

Fish that died less acutely showed the most pronounced lesions. Exophthalmos in one or both eyes was marked, and there was increased pigmentation of the skin. When the eye was opened, the posterior chamber and, to a lesser extent, the anterior chamber were seen to be filled with blood. This resulted in a displacement of the lens, which usually was either of normal size and transparency, or sometimes smaller, degenerated and opaque. The periocular tissues showed massive haemorrhages and oedema. Internally, petechial haemorrhages, as described for the acute cases, seldom occurred. The livers and spleens were enlarged.

Chronic cases often showed normal eyes, while in others the eyes were absent. Empty eye-sockets were filled with connective tissue, overlain with pigmented skin. The degree of pigmentation depended on the degree of blindness. If both eyes were severely affected, the overlying skin was black, but where an amount of vision or light perception remained, the pigmentation was patchy. Where exophthalmos persisted, the eyes were mostly malformed, fibrotic and insensitive to light. In many of these cases the lenses were completely absent.

Gross pathology—Experimental cases

Experiment 1. In these cases the gross pathological observations were limited to peri- and intraocular haemorrhages, periocular oedema and small petechial haemorrhages in the gills and liver. In the case of trout injected intramuscularly, the site of injection appeared haemorrhagic. In the in-contact controls, the intestines appeared inflamed.

Experiment 2. In both the intraperitoneally and intramuscularly injected groups, the livers showed yellow discoloration and focal petechiae. The spleens were enlarged, and the kidneys severely congested.

The injection sites appeared haemorrhagic and in some fish the rectum protruded slightly from the anus. In the in-contact controls, the intestines were also inflamed.

Experiment 3. Trout that were injected intraperitoneally and intramuscularly showed the same gross changes as those indicated in Experiment 1. Exophthalmos, however, was more pronounced in this experiment. The control fish, which were not in contact with experimentally infected fish, failed to show any symptoms.

Experiment 4. Fish that died during the course of the experiment were limited to one *S. mossambicus,* which had been injected intraperitoneally. This fish showed discoloration of the muscles at the injection site and a haematoma in the abdominal cavity. The liver, spleen and kidney were slightly enlarged.

Histopathology

The histopathology of the natural cases did not differ from those of the experimentally produced ones.

Livers of trout from the natural outbreak and Experiments 1, 2 and 3 showed moderate to marked fatty degeneration (Fig. 5). Kupffer cells were prominent, and mononuclear cells, mainly lymphocytes, were scattered in the sinusoids. In some cases the sinusoids were dilated and congested (Fig. 5), whereas in others, congestion only was observed. Occasionally blood vessels and sinusoids were seen to contain a few strands of coccoid bacteria.

In all the cases examined, the spleen was congested (Fig. 9). In 2 cases, however, there were distinct increases in the immature and mature lymphocyte population. Areas of focal necrosis, containing many bacteria, were often seen (Fig. 9 & 10).

Kidneys were mostly haemorrhagic, and some tubular epithelial cells showed degenerative changes.

The heart muscle of several trout showed moderate to severe haemorrhages as well as degeneration of groups of muscle fibres.

The eyes showed the most severe histological changes. Capillaries in the sclera as well as the choroid body were severely congested and haemorrhages occurred frequently. In the less severe cases, haemorrhages occurred in the sclera and from the choroid into the vitreous humor. In severe cases, haemorrhages occurred in the optic nerve and its surrounding connective tissues in the vicinity of the optic disc, and diffusely in the vitreous humor. Large haematomata often occurred between the sclera and the choroid with a resultant detachment of the retina and choroid (Fig. 11–14). To a greater or lesser degree the lenses of many of the eyes examined showed degenerative changes. In severe cases, the lens showed fragmentation of the fibres and contained masses of globular debris. In less severe cases, the lens showed only a number of clefts, which contained degenerated lens substance. Oedema and haemorrhages in the muscles and connective tissue surrounding the eyes occurred frequently (Fig. 13).

Focal vacuolization and necrosis of numerous fibres occurred in skeletal muscles (Fig. 6–8). Small haemorrhages were seen in the interstitium, as well as an infiltration of large mononuclear cells and lymphocytes. Numerous colonies of coccoid bacteria were observed in the muscles of trout that had been injected intramuscularly (Fig. 7 & 8).
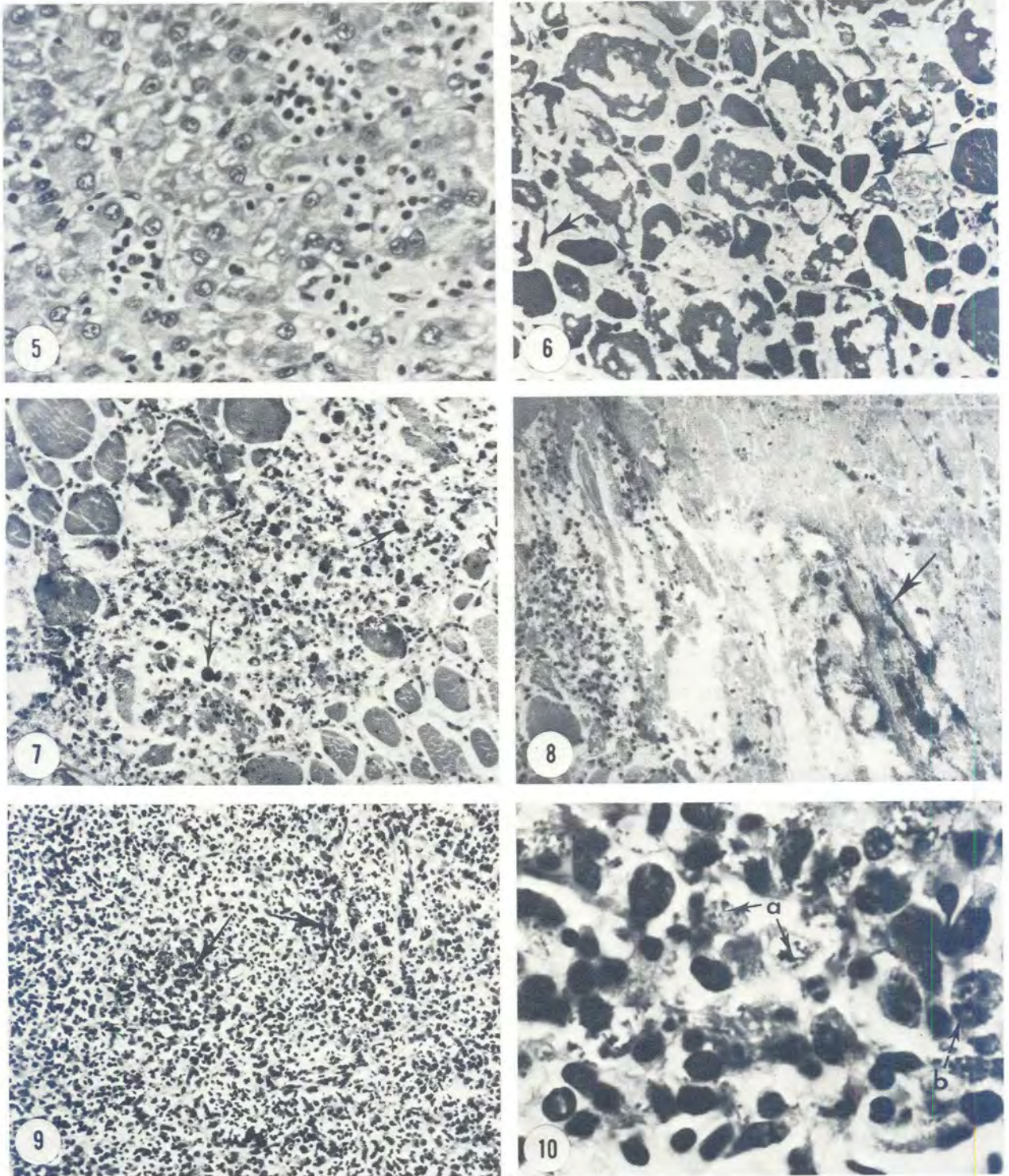
FIG. 5 Liver of rainbow trout showing fatty changes and dilated, congestive sinusoids; HE, ×500
FIG. 6 Degeneration and vacuolization of skeletal muscle. Arrows indicate bacterial colonies; HE, ×200
FIG. 7 Area of necrosis in skeletal muscle. Only a mild round cell infiltration is apparent. Arrows indicate bacterial colonies; HE, ×200
FIG. 8 Advanced necrotic lesion in skeletal muscle. Arrow indicates bacterial colonies; HE, ×200
FIG. 9 Areas of focal necrosis in spleen indicated by arrows; HE, × 200
FIG. 10 Area of focal necrosis in spleen, indicating colonies of bacteria (a) and karyorrhexis (b); HE, ×1200
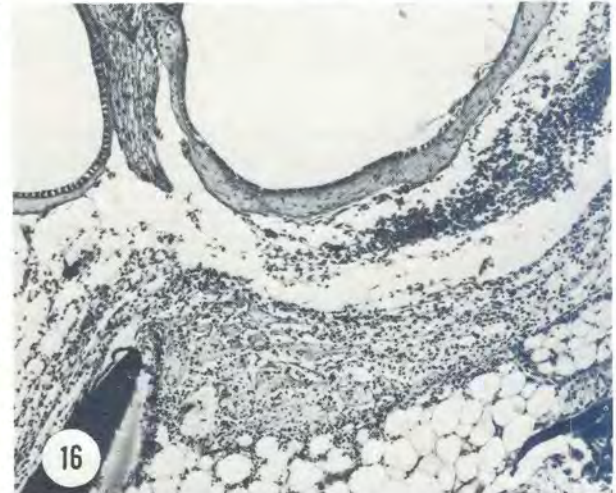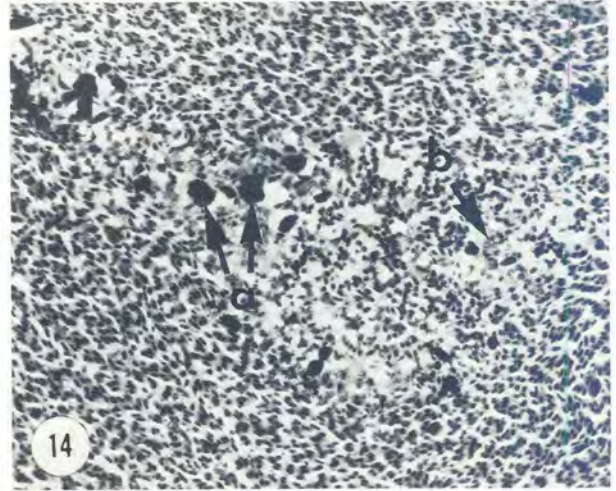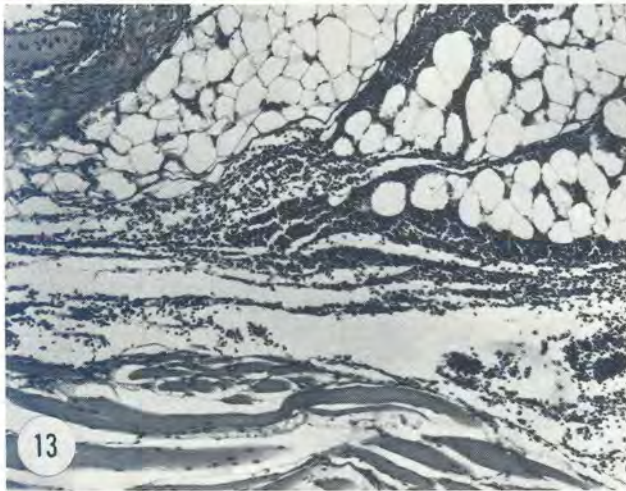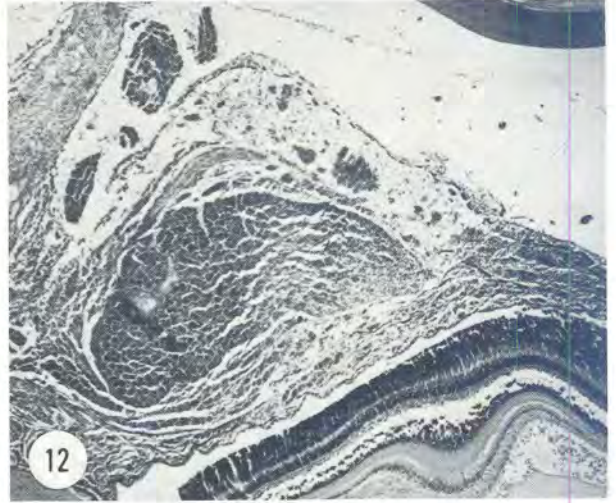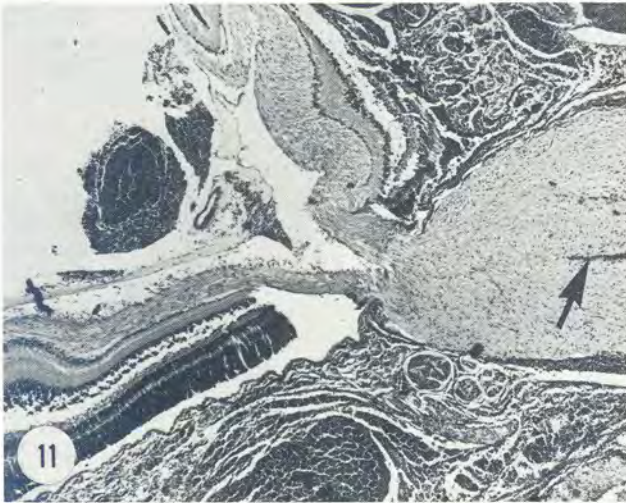
FIG. 11 Eye of trout showing haemorrhage in posterior chamber and optic nerve (arrow); HE, ×30
FIG. 12 Eye of trout, showing massive haematoma in posterior chamber; HE, ×30
FIG. 13 Periocular oedema and haemorrhage in eye of trout; HE, ×75
FIG. 14 Hematoma in eye of trout; bacterial colonies (a) mild influx of macrophages (b) are evident; Giemsa's, ×200
FIG. 15 Patchy meningitis in brain of trout; HE, ×75
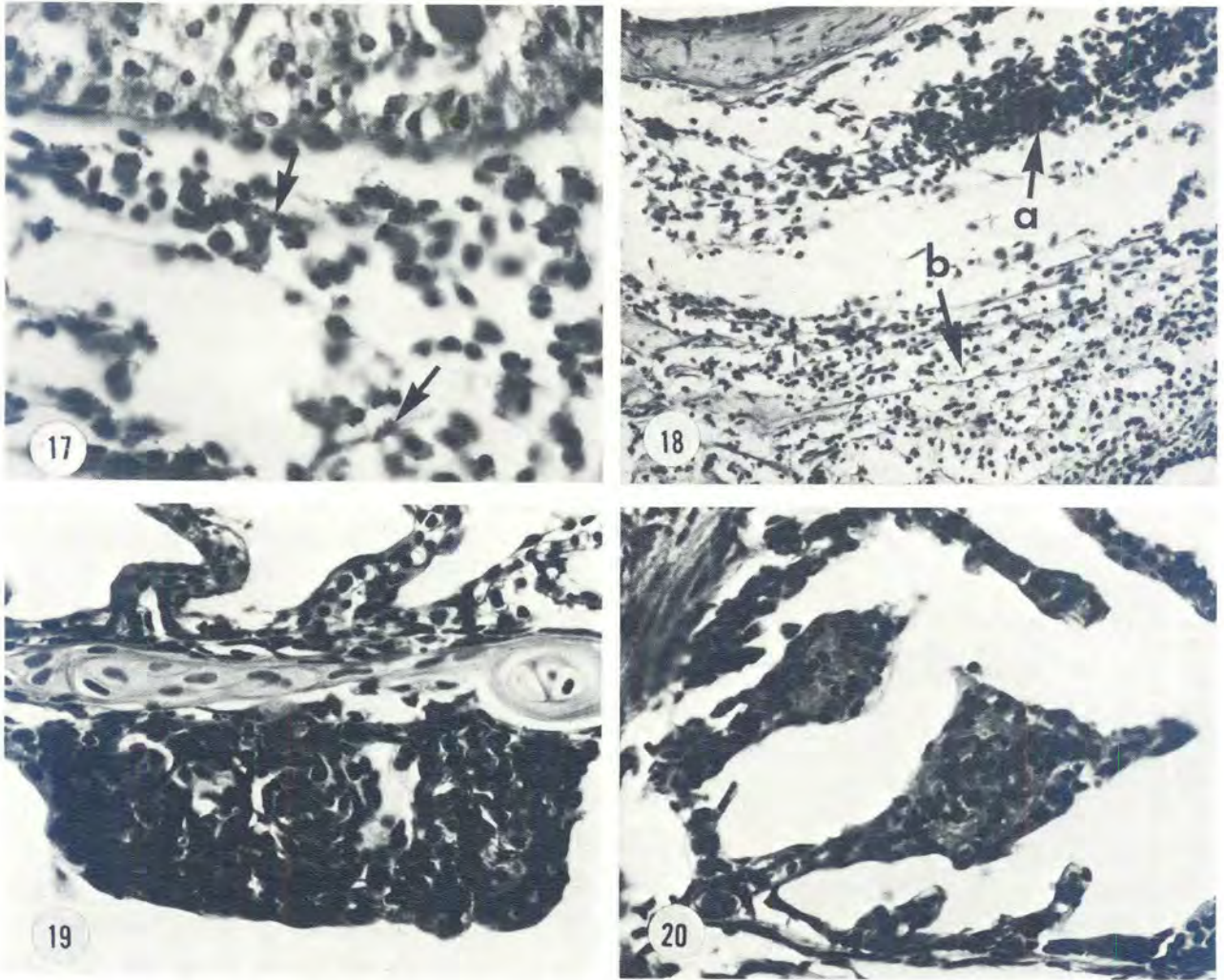FIG. 16 Brain of trout, showing small haemorrhage and mild round cell infiltration of meninges; HE, ×75

FIG. 17 Meninges, showing bacterial colonies (arrows); HE, ×800
FIG. 18 Area of haemorrhage (a) and mild round cell infiltration of meninges (b); HE, ×300
FIG. 19 Gill filaments of which the upper ones are normal. The lower ones show thrombosis, hyperplasia and fusion; HE, ×600
FIG. 20 Thrombosis in gill filaments; HE, ×600

Small petechial haemorrhages were occasionally seen in the gills and in a number of cases the capillary vessels were severely distended. Occasional thrombi occurred in the vessels of the gill filaments, some of which were fused, and the epithelium appeared hyperplastic (Fig. 19 & 20).

In a number of fish, meningitis, patchy in some and diffuse in others, was seen (Fig. 15 & 16). Large numbers of diplococci, both free and packed in macrophages, occurred (Fig. 17). The meningitis was characterized by the infiltration of mononuclear cells (Fig. 18).

In chronic cases, the changes were limited to Kupffer cell proliferation in the liver, organizing haematomata in the muscles, kidney, periocular tissues and the posterior chamber of the eye and lymphoid hyperplasia in the spleen. Sometimes the resulting fibroplasia was so advanced that the eye appeared totally fibrotic. No components other than the cornea, sclera, parts of the choroid and remnants of the retina could be discerned.

The single Mozambique bream showed haemorrhage and disruption of muscle fibres at the injection site. The spleen, liver and kidney showed mild cloudy swelling and mild congestion, but were otherwise normal.

DISCUSSION

From the literature it is clear that only a few cases of streptococcal fish diseases have been recorded.

Baker & Hagan (1942) found that *Streptococcus faecalis* was responsible for mortality in rainbow trout, 3–5 cm long. Hoshina, Sano & Morimoto (1958) also found *S. faecalis* in the heart blood, spleens and kidneys of diseased rainbow trout. They reported severe lesions in the intestines which were characterized by mucosal necrosis and a cellular infiltration in the *lamina propria* and *muscularis*. In the experimental infections (Experiments 1 & 2), the in-contact controls showed hyperaemia of the intestine, but neither necrosis nor cellular infiltrations were evident.

Robinson & Meyer (1966) isolated and identified an organism belonging to the B-haemolytic streptococci from golden shiners (*Notemigonus chrysoleucas*). They found that intra-peritoneal injections of the organism were lethal to golden shiners, bluegills (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*) and American toads (*Bufo americanus*).

Cook & Lofton (1975) undertook pathogenicity studies with a non-haemolytic group B *Streptococcus*, an organism identical with that isolated by Plumb, Schachte, Gaines, Peltier & Carrol (1974), from

marine fishes on the Alabama and Florida coast, as well as 6 streptococci which were not related to fish. Five species of marine fish were used and different numbers of bacteria were injected intraperitoneally. They found that the group B, non-haemolytic *Streptococcus* was highly pathogenic. The Lancefield group D organisms studied by Cook & Lofton (1975) were found to lack the pathogenicity of the group B, non-haemolytic streptococci. In the present experiments, described above, it was found, however, that the Lancefield group D organism proved 100% fatal to trout, but that bream, banded bream, carp and largemouth bass were refractory to the infection.

From the natural outbreaks and the experimental infections it was seen that the main symptoms were uni- or bilateral exophthalmos, hyperpigmentation of the skin, lethargy, erratic swimming and eventual death. The most consistently found lessions were haemorrhages in and around the eyes, the visceral organs and the sub-peritoneal abdominal wall.

Both the gross- and histopathology showed the changes usually associated with a haemorrhagic septicaemia, in the acute, subacute and chronic forms (Smith, Jones & Hunt, 1972).

Wolke (1975) describes epidermal lesions in rainbow trout and golden shiners. Dermal lesions, which were also described by Robinson & Meyer (1966) in golden shiners, have the same distribution as those reported by Baker (as cited by Wolke, 1975) in trout. Dermal lesions have not been observed in natural cases or experimental infections in rainbow trout, infected with the group D organism.

Focal necrosis of the kidney and liver, renal tubular epithelium degeneration, fusion of gill lamellae and, in some cases, skeletal muscle degeneration, as described by Hoshina *et al.* (1958) and Wolke (1975), were also seen in these experiments and natural outbreaks.

Trout is an introduced species in South Africa and can only be farmed in those areas where the water temperatures are constantly low. Because the temperature of the water on the first farm was constantly 19 °C, which approximates the upper limit for trout, the fish grew fast and could conceivable have been subjected to stress. Outbreaks on the other farm occurred in summer, when water temperatures were high, and subsided as soon as the water temperatures dropped in late autumn. Overcrowding and other forms of stress might have aggravated the condition, especially with water temperatures fluctuating around 20 °C. This appears to be a problem associated with intensification of which the source and significance are still to be determined.

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