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Clinical and pathological observations on *Streptococcus* sp. infection on South African trout farms with gas supersaturated water supplies

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ABSTRACT

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Infection with a faecal *Streptococcus* species belonging to Lancefield group D has been a major cause of mortalities on certain trout farms on the escarpment of the Mpumalanga Province in the Republic of South Africa. Evidence is given of a connection between subclinical gas bubble disease and infection with this organism in rainbow trout, *Oncorhynchus mykiss*. The condition causes serious losses during the summer months when water temperatures rise above 18 °C. The larger table-size fish are affected first and present with exophthalmos before developing signs of a typical bacterial septicaemia. The design of the water supply to the fish ponds on these farms allowed supersaturation of the water with atmospheric gas. Vortex formation at water extraction points appears to have been the main cause of air entrainment into the water supply leading to the ponds. By correcting the faults responsible for the development of hyperbaric gas pressure in the water, it was possible to control this disease on two badly affected farms. The overlapping similarities in the clinical signs and pathology of gas bubble disease and that seen in fish suffering from *Streptococcus* D infection are discussed, as well as the diagnostically significant changes in the gill tissues that are specific to subclinical exposure to gas supersaturation in the water. An explanation is given for the increased susceptibility to infection with this organism.

Keywords: Embolism, exophthalmos, gas bubble disease, gas entrainment, *Onchorhynchus mykiss*, *Streptococcus* D, supersaturation, trout

INTRODUCTION

Infection by a faecal *Streptococcus* sp. belonging to the Lancefield D group has periodically been held responsible for significant fish losses on certain South African trout farms (Bragg & Broere 1986). The condition was first described by Boomker, Imes, Cameron, Naudé & Schoonbee (1979) in rainbow trout, *Onchorhynchus mykiss*, on a farm in the Magaliesberg district (North West Province) of South Africa. The water source for this farm was from a dolomitic spring a short distance upstream from the farm ponds. Another fish farm further downstream has been successfully producing Koi carp, *Cyprinus carpio*, since the early 1980s without experiencing this problem. Bragg, Todd & Lordan (1988) isolated a similar *Streptococcus* sp. from mud and a dead fresh-water crab on a site with a history of streptococcus infection in trout. A *Streptococcus* sp. biochemically and serologically identical to the trout pathogenic species was isolated from homogenized samples of the leech, *Batracobdelloides tricarinata* (Blanchard), from a dam far removed from trout

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waters (Bragg, Oosthuizen & Lordan 1989). This species of leech has never been recorded to feed on rainbow trout, and the authors concluded that the *Streptococcus* sp. pathogenic to rainbow trout may not be an obligate pathogen of these fishes. A similar *Streptococcus* sp. has been reported from Australia with mortalities in excess of 60% in affected rainbow trout (Carson, Gudkovs & Austin 1993).

During the mid 1980s many South African trout farms experienced lower than normal water flows due to a fairly protracted drought. Outbreaks of *Streptococcus* D infections persistently occurred on the same farms each summer. Other similarly managed farms in the area did not experience the problem. The first signs of the disease started in October when water flows were at their lowest and average daily water temperature remained above 18 °C. Peak morbidity and mortality occurred in mid to late summer. The larger fish appeared to be most susceptible and the greatest losses occurred in near market sized fish. By the end of March morbidity and mortality dropped, as average daily water temperatures fell below 18 °C. The typical clinical signs of uni- and bilateral exophthalmos led farmers to refer to this condition as "pop-eye".

Trout farms on the eastern escarpment of the South African highveld often produce fish in areas of high ambient summer temperatures and rely on relatively small mountain streams which retain cooler water temperatures during the rapid descent down the escarpment. Some farms experience summer water temperatures of 25 °C and higher. The occurrence of *Streptococcus* D infection showed no correlation to excessive summer water temperatures experienced on comparable farms and also did not appear to spread from one farm to another, although the disease appeared to be highly infectious within a problematical farm.

Gas bubble disease is a well documented non-infectious, physically induced process caused by exposure of fish to uncompensated, hyperbaric total dissolved gas pressure in the water. Primary lesions are produced in the blood vessels (emboli) and in tissues (emphysema) leading to subsequent physiological dysfunctions (Bouck 1980; Weitkamp & Katz 1980; Roberts 1989). Protracted exposure to high partial pressures may lead to the development of necrosis and infection in the emphysematous sites (Bouck 1980). Long term chronic exposure often leads to eye lesions associated with the development of exophthalmus ("pop-eye") (Bouck 1980; Krise & Smith 1993; Machado, Garling, Kevern, Trapp & Bell 1987). Colt (1986) distinguishes between the acute and chronic effects of gas supersaturation exposure. The chronic effects of sublethal gas supersaturation levels that may be present on fish farms are not well identified due to the low levels of gas supersaturation involved, a delay of 1–2 months before the start of mortality, a lack of typical clinical signs of gas bubble trauma, and significant seasonal variation in the gas levels of many surface waters (Colt 1986).

Gas bubble trauma can be produced by inflowing water that is supersaturated or by production of gas supersaturation within the fish farm. Injurious agents such as gas emboli, immune complexes and infectious organisms are easily caught up in capillary networks such as the choroidal rete and may lead to exophthalmos (Engelman, Collier & Marliave 1984). Gas bubbles localizing within the choroidal rete alter vascular permeability resulting in eye pathology. Possible mechanisms of gas embolus formation include malfunctioning of the oxygen-concentrating mechanism of the choroidal rete, exposure to water supersaturated with atmospheric gases or rapid decompression (Engelman *et al.* 1984). Smith (1988) describes gas emboli in the adipose tissue posterior to the choroid, within the choroid or between the choroid and retina, in fish with exophthalmos experimentally exposed to gas supersaturation. Thrombosis in the blood vessels of the gills is commonly associated with gas embolism.

The disease known as *Streptococcus* D infection is described on the two largest table fish producing rainbow trout farms on the eastern Mpumalanga Province escarpment. Evidence of subclinical gas bubble disease and the attempts to exclude gas supersaturation from the water supplies of these farms is discussed in relation to the control of this disease.

MATERIALS AND METHODS

During the period from January 1986 to November 2000, observations were made and tissue sampling done on trout farms on the eastern escarpment of Mpumalanga Province, Republic of South Africa. Investigations included assessment of the design and management of the farm, examination of mortality records, and clinical and post mortem examinations of affected fish. In addition, farmers submitted either live or freshly dead fish for diagnostic purposes. Post mortem examinations included the microscopic examination of skin scrapings and wet mount gill preparations, as well as the collection of

tissue samples for bacteriological and histological examination. Water quality criteria were established routinely during the early visits to the farms.

Wet mount gill preparations were made by removing 6–12 gill filaments (primary lamellae) from random gill arches with a fine pair of scissors, gently dispersing them in a drop of water on a microscope slide and covering them with a cover slip. Microscopic examination of the secondary lamellar capillary network was done at 100x, 200x and 400x magnifications.

Bacteriological samples were taken aseptically from the kidney and liver of sacrificed fish with obvious lesions, and were submitted to the Onderstepoort Veterinary Institute on V-pak transport medium swabs. Bacterial isolation was done on blood tryptose agar at ambient temperature above 18 °C. Tissue samples of gill, liver, kidney, spleen and pyloric caeca were collected from sacrificed fish showing obvious signs and were fixed in 10% neutral buffered formalin. After being embedded in paraffin wax, 5 µm thick microtome sections were cut and stained with haematoxylin and eosin.

RESULTS

Farm 1 is situated on the Lunsklip River and had an annual production of up to 150 tons of rainbow trout. Prior to 1993 annual mortalities ascribed to infection with *Streptococcus* D in grow-out fish were between 15 and 20 %. The most serious mortalities were experienced in the larger, near market size fish. In 1993 the farmer reported mortalities in excess of 70 %. During the period of this study no further cases of *Streptococcus* D infection were reported on this farm after the winter of 1994.

Farm 2 is situated on the Elands River and had an annual production of up to 80 tons of rainbow trout. Initially, annual losses in grow-out fish ascribed to *Streptococcus* D infection were in the region of 20 %. In 1998 mortalities in excess of 70 % were reported. During the summer of 1999 and 2000 the farmer ascribed no losses to *Streptococcus* D infection.

System design and hydrological observations

The water take-off for Farm 1 was from a weir situated approximately 350 m downstream from a 216 m high waterfall. The waterfall descended via a series of steep rocky cascades before entering a large pool. Water was extracted via a pipe from the bottom of the weir approximately 1.3 m below the weir overflow. Water flow from the weir was controlled by a stop-valve at the extraction point from the weir. A 330 mm diameter pipe of 400 m length, down a gradient of 1 in 66, fed water into the circular on-growing ponds. The pipe was of a thick PVC material and the various sections were joined by heat welding. Production ponds consisted primarily of shallow (1 m deep or less) circular cement ponds with conical bottoms. The main production ponds varied in size from 37–87 m^3.

Water was fed into each pond by a smaller pipe through a fully open stop-valve at the discharge point of the pipe, and water was only used once before being discharged, via large earth ponds, back into the river. A short distance down river water was again extracted from it into a header canal and fed via individual pipes into five shallow earth ponds of 40 m^2 surface area.

During periods of low water flow the entire river was fed through the farm. The water level in the weir was maintained by partially closing the stop-valve at the base of the weir thus reducing the flow in the pipe. The farmer reported that the gravitational pull created in the pipe was sufficient to cause partial collapse of the pipe. When the water level in the weir dropped to 1 m and less above the take-off point for the pipe, a strong vortex was present on the surface of the water in the weir allowing air to be sucked into the pipe. The likelihood of further air entrainment into the water supply via the stop-valve or small venturi-type leaks in the pipe joins was considered. In order to avoid air being sucked into the supply pipe it was decided to keep the weir stop-valve fully open at all times and rather to maintain the water level in the weir via the small stop-valves at the inflow of each pond. This allowed a positive pressure to be maintained in the entire pipe system feeding the ponds and prevented the formation of a vortex on the weir surface. From this time onward the prevalence of *Streptococcus* D infection declined dramatically.

Farm 2 consisted of two separate units on the same river approximately 5 km apart. Cement production ponds on the top unit were of circular design, with conical bottoms, less than 1 m deep. Pond volumes varied from 47–135 m^3. The water supply was taken via an open canal from a weir in the river approximately 350 m upstream of the farm. The canal followed a gentle gradient down to the farm with minimal turbulence in the canal water. Water was led from the canal into small header boxes, situated in the side of the canal (one per pond). Short lengths of pipe (from 1–4 m) fed the

water into each individual pond. Water was used only once except in severe drought conditions when it was pumped back from the river downstream of the farm into the canal. At the time of the initial investigation, water flow, into each pond, was controlled by a stop valve at the discharge point to the pond. Vortex formation in the majority of header boxes allowed air entrainment into the water supply pipes to the ponds (Fig. 1). It was suspected that cavitation in the stop valves may have contributed to gas supersaturation. In order to prevent these occurrences the stop valves were replaced by pipe elbows which could be rotated to raise or lower water flow. The header boxes were supplied with polystyrene floats which appeared to eliminate the vortex formation.

Following these changes the prevalence of *Streptococcus* D infection significantly declined for a num-

FIG. 1 Typical vortex in a header box showing air entrainment (arrow) into a pipe

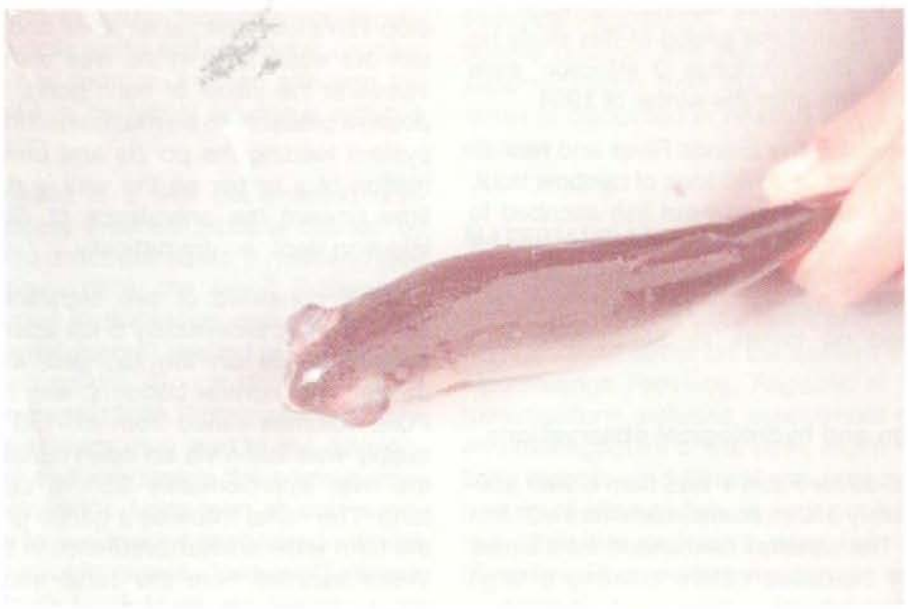

FIG. 2 Exophthalmos in a rainbow trout suffering from *Streptococcus* D infection

ber of years but in the summer of 1998/1999 renewed outbreaks of the infection occurred on this farm. Investigation revealed the presence of several additional points of air entrainment. High summer water flow into the canal was controlled by a large metal blade sunk to just below the surface of the canal. This blade controlled the water flow into the canal and prevented flooding of the canal and farm during times of peak water flow. Vortex formation was evident behind the blade Lifting the blade and maintaining it above the water during normal water flow times reduced the prevalence of the disease to some extent. Further enquiries revealed that a large dam had recently been constructed across the river approximately 2 km upstream of the farm and a large pipe in its wall discharged water into the river on a continuous basis allowing vortex formation in the water at the surface of the dam. At the beginning of the summer in 1999/2000 the metal blade was entirely removed from the take-off weir and the owners of the dam upstream were requested to keep the discharge pipe in the dam wall closed and to allow all the water to spill over the wall. No cases of *Streptococcus* D infection were reported during the summer of 1999 and 2000.

The second production unit on this farm was situated approximately 5 km downstream of the first on the same river. The ponds were earthen and rectangular with a volume of 400 m^3 each and a maximum depth of 2.3 m. Water was extracted from a weir in the river and flowed freely down a 1 km long open canal. Each pond received water via a concrete chute situated in the side of the canal. At the take-off point from the weir, water entered the canal through a rectangular gap in a wall supporting a sluice blade across the canal opening. The upper horizontal margin of this gap was a few centimeters below the water surface when the blade was fully lifted to allow maximum water flow down the canal. Approximately half way down the length of the canal the water flowed through a similar gap in a wall constructed across the canal to keep logs and debris from reaching the fish ponds during times of high flow. Vortices, allowing air entrainment, were visible on the surface behind both of these walls. Due to the serious structural changes required to eliminate this problem it was decided to introduce three pairs of paddle wheel aerators into the canal downstream of the last wall. The pairs rotated in opposite directions so as not to dam back the water in the canal. An immediate decrease in the mortality rates from above 130 fish per day to less than 20 was reported by the farmer, without the use of antibiotic medication. During the summer 1999 and 2000 no antibiotics were needed to control *Streptococcus* D infection on this farm.

Water quality and stocking density

Basic water quality parameters determined on both farms were as follows: Farm 1: pH 8.7; hardness 68.4 mg/ℓ; alkalinity 14 mg/ℓ as CaCO3. Farm 2: pH 9.0; hardness 105.6 mg/ℓ; alkalinity 34.2 mg/ℓ as CaCO3. No equipment was available at the time to measure dissolved gas pressures. The presence of gas emboli visible in the gill vasculature on wet mount microscopic preparations was used as an indicator of dissolved gas pressures above saturation level. Both farms were stocked at levels not exceeding 0.7 ℓ/min water flow per kg of live fish.

Clinical observations

Exophthalmos, both unilateral and bilateral, was the first clinical sign of an impending *Streptococcus* D outbreak to be noticed by the farmers on both farms (Fig. 2). Outbreaks occurred seasonally between October and March, with increasing morbidity and mortality towards the end of March. Early cases with exophthalmos were observed feeding and still appeared to ingest food. Fish with eye lesions became progressively blind and the skin colour darkened. Increasing numbers of blind fish were seen swimming lethargically near the sides of the ponds as mortalities started to rise. Post mortem examinations of the fish revealed in most cases a turgid swollen body. The protruding eyes often contained haemorrhages within and around the eye, and perforation of the cornea was observed in some cases. Petechiae and ecchymoses were a consistent finding in the livers of affected fish. Haemorrhages were also present in the peritoneum of some cases. The kidneys appeared moderately swollen. The stomach and the intestines were often found to be full of ingesta. Food withdrawal from affected ponds indicated that the presence of ingesta in the stomachs of moribund fish was due to a retarded gastric emptying time rather than from agonal food ingestion.

Microscopy of wet mount gill preparations

Emboli of gas (Fig. 3) were consistently found in the vasculature of the secondary lamellae of the gills of all fish examined with exophthalmos. Apparently healthy fish without lesions, sampled from amongst a population of infected fish, only occasionally showed emboli in the gills. Microscopic examination of wet mount gill preparations illustrated the progression of gas embolus lesions

in 3 stages. The first stage revealed clear gas emboli (Fig. 3) evident particularly near the distal end of the primary lamellae and restricted to only one half of the capillary loop plexus. Gas emboli appeared to follow the branched nature of the gill arterioles of the secondary gill lamellae. In some cases the emboli could be observed extending down the length of one gill arteriole for varying lengths. The second stage involved the presence of a small gas embolus in the arteriole, formation of a distinct thrombus distal to the embolus (Fig. 4) and ischaemia for a varying distance distal to the thrombus. The third stage consisted of a darkly pigmented lesion taking on the shape of a gas embolus in the secondary lamellae and appeared to represent melanization of a resolving thrombus (Fig. 5 and 6).

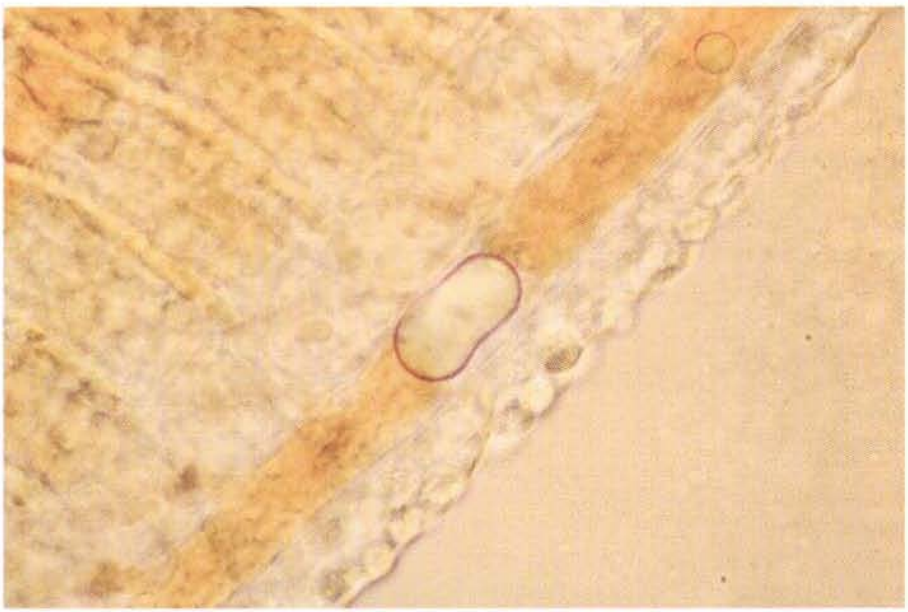

FIG. 3 Gas embolism in a gill arteriole as seen in a wet mount gill preparation (x 2 000)

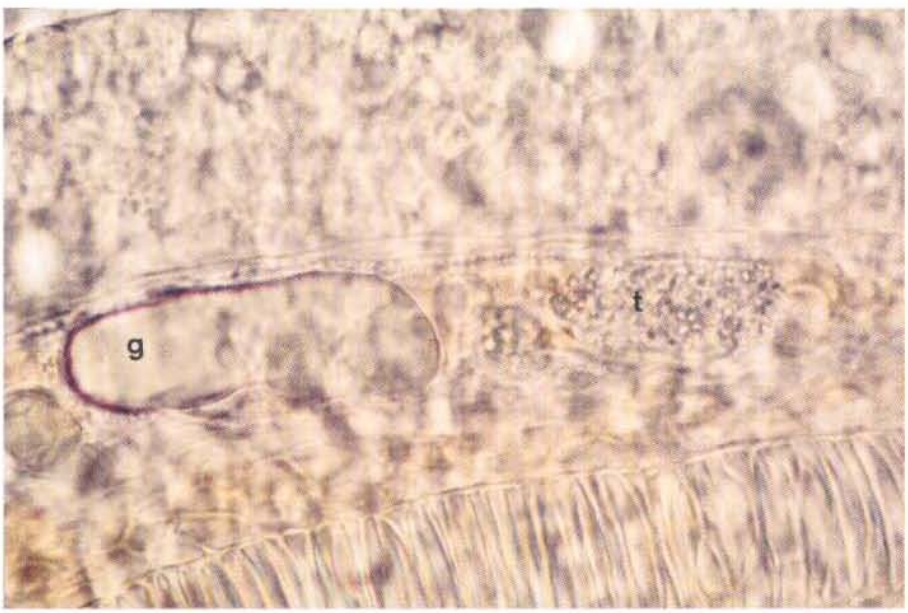

FIG. 4 Gas embolus (g) in a gill arteriole showing formation of a thrombus (t) Gill wet mount preparation (x 2 000)

Histopathology

Lesions were consistent in all fish with exophthalmos, and were typically visible in the liver, kidney and spleen.

Large irregular foci of haemorrhage and necrosis were apparent in all of the affected livers. Hepatocytes showed varying degrees of vacuolar and fatty degeneration. Foci of inflammatory round cell infiltration were evident surrounding bile duct tracts and the hepatic blood vessels. Sinusoids often appeared congested. In sections from some fish a mixed inflammatory exudate was present surrounding the liver and pancreatic tissues indicative of a peritonitis. Colonies of coccoid bacteria were sometimes visible within the foci of haemorrhage

FIG. 5 Melanin being deposited at one end of a resorbing gas embolus (g) in a gill arteriole. Gill wet mount preparation (x 2 000)

FIG. 6 Melanized remnants of thrombi caused by gas emboli in the distal ends of gill arterioles. Gill wet mount preparation (x 1 000)

and necrosis in the liver (Fig. 7). Thrombi were occasionally seen in hepatic blood vessels. The spleens were generally congested and contained necrotic cells associated with the white pulp. In the kidney, the tubules were intact and the lining epithelium unaffected, and lesions appeared to be restricted to the renal interstitium which appeared more loosely structured than normal due to interstitial oedema. Necrotic cells were consistently evident dispersed throughout the renal interstitium. Changes observed in the gills were rather inconsistent and variable, although thromboses in the gill capillaries associated with rupture of the pillar cells were common as were hypertrophy and varying degrees of mild hyperplasia of the secondary lamellar epithelium. Empty spaces indicative of the presence of gas emboli could occasionally be seen in the gill arterioles (Fig. 8).

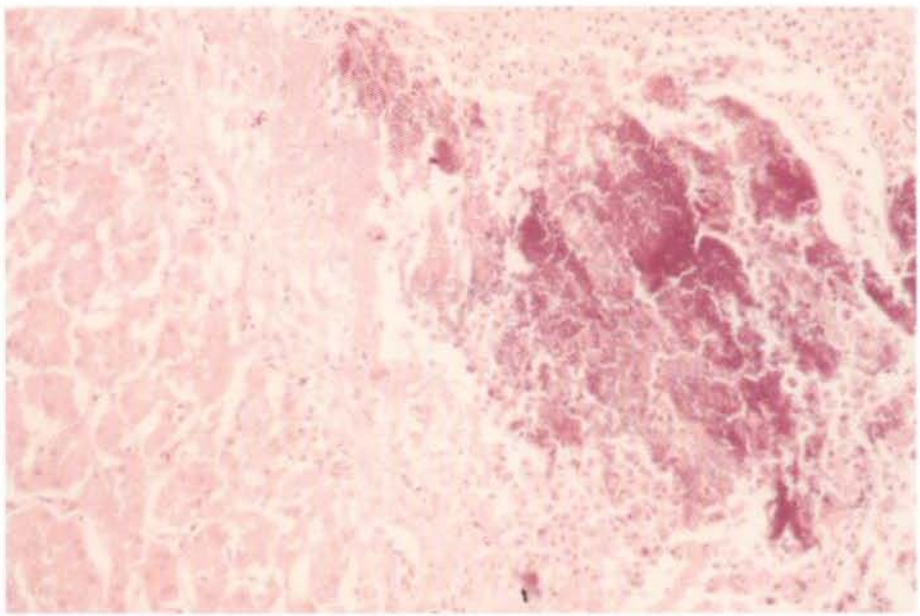

FIG. 7 Liver showing area of necrosis and haemorrhage around a colony of coccoid bacteria. (HE, x 1 000)

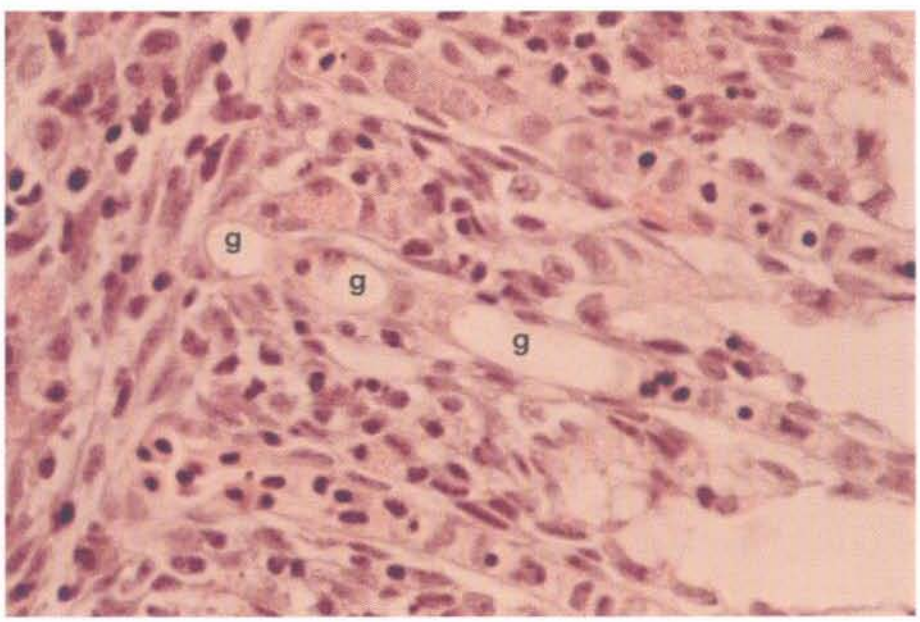

FIG. 8 Gill cross section showing intra-vascular air bubble spaces (g) at the base of a secondary gill lamella. (HE, x 2 000)

Bacteriology

Bacteriological examination of the livers and kidneys of fish with exophthalmos repeatedly resulted in the isolation of *Streptococcus* Lancefield group D organisms. These cultures were almost always sensitive to oxytetracycline. Occasionally *Aeromonas hydrophila* was isolated from the same swabs together with the *Streptococcus* D. Bacterial gill disease with associated myxobacterial lesions was the only other significant bacterial disease to occur on these farms during this period. In the laboratory the *Streptococcus* D cultures did not grow at temperatures below 18 °C.

DISCUSSION

The most consistent clinical sign of infection with *Streptococcus* D in trout is the development of exophthalmus and other serious eye lesions. Similar eye lesions are described in outbreaks of streptococcal infection in cultured turbot (Doménech, Fernández-Garayzábal, Pascual, Garcia, Cutuli, Moreno, Collins & Dominguez 1996) and in tilapia and channel catfish (Chang & Plumb 1996). Almost identical eye lesions in the absence of bacterial infection have been recorded when fish have been exposed to water supersaturated with gases (Krise & Smith 1993; Roberts 1989). The consistently repeated identification of gas emboli in the gill vasculature of fish showing signs of *Streptococcus* D infection in the present investigation indicated a significant clinical correlation between the presence of gas emboli and infection with this bacterium. The same prevalence of occurrence of gas emboli in the gill vasculature was also apparent in fish with *Streptococcus* D infection from various other farms in the Mpumalanga Province.

Entrainment of air into pressurized water systems is probably the most common mechanism for the production of gas supersaturation on fish farms (Colt 1986). As on the two farms in question this may have occurred at the intake, at water control structures or at locations within the water systems with subatmospheric pressures. Many of these processes occur seasonally, during specific times or in specific parts of the fish farm (Colt 1986). Exposure of fish to moderate gas supersaturation levels may require several months of exposure before mortality is observed. This may make it difficult to determine that gas supersaturation in fact is the cause of the mortality and the source of the gas supersaturation is often difficult to pinpoint (Colt 1986). The improvement in the health of the fish and the reduction in the prevalence of *Streptococcus* D infection were dramatic, once air entrainment was prevented in the water supply line on the two fish farms under discussion.

Gas embolism in fish is an environmentally induced process caused by uncompensated, hyperbaric, total dissolved gas pressure in the water supply. Resulting lesions are dependent on the level of supersaturation, duration of exposure and the species and life stage of the fish involved (Bouck 1980). Diffusion of gas into the swim bladder, as into an existing bubble, will occur at any total gas pressure above 100 %, whereas bubble formation may require a higher threshold being influenced by the surface tension of the blood, number and size of nucleation sites for bubble growth, compliance characteristics of the vascular system, gas diffusion rates through the animal's integument, and functional properties of the swim bladder (Colt 1986). In human decompression illness, it is thought that the invasion of extravascular bubbles into ruptured capillaries provides the nuclei for the formation of bubbles within the blood stream (Moon, Vann & Bennett 1995). Provided that bubbles remain in the venous blood stream they would be filtered out by the human lung vasculature and remain fairly harmless (Moon *et al.* 1995). Exophthalmia in salmonids has been associated with gas embolism and typically follows chronic exposure to low levels of supersaturation (Bouck 1980). Fish with gas bubble-induced exophthalmia can and do feed readily, indicating that their vision may remain functional and effective (Bouck 1980). A consistent finding on the farms containing fish suffering from *Streptococcus* D infection was that exophthalmic fish in the initial stages of the disease were observed to take feed.

The United States Environmental Protection Agency has perpetuated the critical level of 110 % total gas pressure, set by the National Academy of Sciences (Water Quality Criteria, 1972, in Weitkamp & Katz 1980), as a water quality standard. Colt (1986) views this criterion of 110 % of barometric pressure or 76 mm Hg as inadequate to protect salmonids exposed to chronic gas supersaturation. The uncompensated differential pressure decreases by approximately 74.3 mm Hg/m if the differential pressure and temperature are uniform with depth (Colt 1984). Fish in most waters that are likely to be supersaturated assume a depth distribution adequate to compensate for supersaturation well above 110 % total gas pressure. Lund & Heggberget (1985) found that rainbow trout do not avoid

air-supersaturation levels from 115–125 % total gas pressure by active hydrostatic pressure compensation (sounding). An incidental type of hydrostatic pressure compensation may explain the observed difference in tolerance to supersaturation between wild fish and fish kept in experimental tanks (Lund & Heggberget 1985). Compensatory depth distribution is withheld from fish in shallow aquaculture ponds. In the case of the deeper earth ponds on Farm 2 the high summer water temperatures may have forced the fish to remain near the surface and the inflowing stream of water to meet their oxygen requirements. This may have resulted in an inadequate depth distribution to compensate for the supersaturation level in the inflowing water.

The effect of chronic gas bubble disease on increased susceptibility to infection is probably underestimated. Weitkamp & Katz (1980) have extensively reviewed the available literature on gas bubble disease. The cause of death in acute cases is generally attributed to anoxia resulting from stasis of the blood. Chronic exposure to lower levels of supersaturation can lead to death from other causes (Weitkamp & Katz 1980). Only limited reference is made by these authors to pathogenic bacteria and secondary fungal infection. Formation of gas bubbles in the tissues and organs may lead to petechiation, restricted blood flow, necrosis and increased mortalities due to secondary bacterial infections such as *Aeromonas hydrophila* (Colt 1986) and *Vibrio alginolyticus* (Elston 1983). Saeed & Al-Thobaiti (1997) reported heavy infections with monogenetic trematodes in Nile tilapia (*Oreochromis niloticus)* after exposure to gas supersaturation During the current study the level of supersaturation in the water was never severe enough to produce visible tissue emphysema and it appears that compromised circulation could affect tissues sufficiently to allow overwhelming secondary infections with *Streptococcus* D to develop. In the absence of secondary infection, fish with subclinical gas bubble disease may well recover when exposed to equilibrated water.

The histological lesions typical of gross gas bubble disease described by Machado *et al.* (1987) in a high morbidity, high mortality trial suggest that the origin of the gas emboli in the gills is from the afferent side of the gill arches. This would imply that the gill vasculature acts as a trap for circulating gas emboli and that these emboli may also be trapped in other capillary networks throughout the body with similar pathological consequences to those observed in the gills Unilateral exophthalmos, a common gross lesion of gas bubble disease, resulted from a space forming between the capillary layer of the choroid and the pigmented epithelial layer of the retina. The earliest histological lesion described by Machado *et al.* (1987) was an emphysematous space within a vessel of the choroid body of the eye.

In the present study gill lesions appeared to be caused by gas displacement of the blood from the afferent arteriole within the gill filaments. Small emboli trapped within the blood vessels of the secondary gill lamellae were observed to result in thrombus formation. These thrombi, although more prevalent near the tips of the primary lamellae, could be observed in the secondary lamellae along the length of the primary filament. Part of the resorption of the thrombus appeared to involve the deposition of melanin into the thrombus leading to irregularly shaped black bodies visible within the gill vasculature on wet mount microscopic examination. The presence of these melanized thrombi indicated chronic exposure to subclinical levels of supersaturation. The examination of wet mount gill preparations was found to be an easy and reliable diagnostic tool in identifying exposure of the fish to gas supersaturation in the water.

The histological changes observed in the fish suffering from *Streptococcus* D infection were typical of a severe bacterial septicaemia. Smith (1988) describes the histological changes associated with gas embolism and emphysema formation resulting in necrosis, inflammation and plasma leakage. He observed gas emboli histologically in the blood vessels of the gill filaments, in postorbital haemorrhages, in the atrium, ventricle and bulbus arteriosus of the heart. In the present study it appears that the subclinical presence of gas emboli and their effects on the capillary vasculature were a significant underlying cause in increasing the susceptibility of the fish to the pathogenic effects of the *Streptococcus* D organism. Typical observations on both farms were that fish, with signs of *Streptococcus* D infection, were always found to have gas emboli in the gill tissues. Apparently healthy fish from the same population seldom had demonstrable gas emboli in the gills. This finding is consistent with the results of bioassays described by Weitkamp & Katz (1980) indicating considerable individual differences in the tolerance of fish to supersaturation.

The dual effect of high summer water temperature on the growth of the *Streptococcus* D organism and on the increasing development of supersaturation in the farm waters, with resultant gas embolisms in fish, appears to have been a predisposing circum-

stance for the pathogenicity of this organism. After the source of gas supersaturation was identified on Farm 1, in 1994, no further cases of *Streptococcus* D infection occurred during the study period and it became clear that in the absence of gas superstauration the *Streptococcus* D bacterium no longer posed a threat to the health of the fish. The situation on Farm 2 was not as easily resolved due to the difficulties in executing structural changes on the header canal and feeder boxes of the upstream production unit as well as the long-term consequences of the dam construction a short distance up river from the take-off weir to the farm. It is clear that hyperbaric gas pressures can exert their influence for considerable distances downstream from their source where the water flow has been slowed down by weir construction. Weitkamp & Katz (1980) reported that even rapidly flowing turbulent streams do not necessarily provide rapid equilibration of dissolved gases. However, improved management of the supersaturation problem on both the production units on Farm 2 have led to a significant reduction in the prevalence of gas embolism in the gills of fish from this farm and to the disappearance of *Streptococcus* D infection during the summer of 2000/2001.

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