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INTRODUCTION

This report consists of a thorough, systematic search of the available publications concerning Enteric Redmouth Disease (ERM) in fish. Also included are the collated verbal opinions of commercial trout farmers gathered during a meeting with two of the authors, R. Edward Larson and George W. Klontz.

The report forms the basis from which current research efforts are operating to provide a detailed description of ERM, and to test and apply, under field conditions, efficacious control methods.

DEFINITION

Enteric Redmouth Disease (ERM) is an acute to chronic systemic bacterial disease of salmonids, particularly rainbow trout. The causative organism is a Gram negative, peritrichous flagellated rod. The acute course of the disease is characterized by reddening in and about the oral cavity, about the vent, and at the base of the pectoral, pelvic, and anal fins, which are often markedly frayed. Ascites typically occurs. In the chronic infection the fish are dark, lethargic, and commonly show bilateral exophthalmia.

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HISTORY

Enteric Redmouth Disease was first recognized as a serious fish health problem in the Hagerman Valley of southern Idaho in the early 1950s. In 1954, Rucker first isolated the causative organism from an epizootic in rainbow trout at the Hagerman National Fish Hatchery.

Confusion and uncertainty, regarding the taxonomic classification of the pathogen, delayed publications on the organism for the next 12 years. During that time the incidence and geographic range of the disease increased greatly. In 1966, Ross et al. published a complete description of the causative organism and assigned the name RM Bacterium pending further taxonomic studies. Rucker (1966) listed the clinical signs of the disease as well as providing information on the epizootiology, treatment, and prophylaxis for the disease.

In the early 1960s, Ross and Klontz (1965) at the Western Fish Disease Laboratory, conducted investigations on the development of an effective oral immunizing agent to protect rainbow trout from epizootics of this disease.

Since the discovery of ERM in southern Idaho, outbreaks of this disease have occurred throughout the western United States with sporadic outbreaks reported in the midwestern and eastern United States and Canada. Today the disease is regarded as a serious threat to trout production in Idaho, and renewed efforts have begun to describe it in detail and to control it effectively.

ETIOLOGY

Classification

The etiologic agent of ERM disease has been placed within the family Enterobacteriaceae. To date, the organism still has not been assigned a genus and species classification. As a result, it is still referred to as RM Bacterium, as suggested by Ross et al. (1966), who orginally described the pathogen.

The name of the disease has changed several times since it was first studied. Originally the disease was referred to as redmouth disease, red vent disease, and bacterial hemorrhagic septicemia. These terms were confused with the redmouth disease caused by *Aeromonas hydrophilia*, a member of the *A. liquefaciens* complex, so McDaniel (1971) proposed the disease be referred to as Hagerman Redmouth Disease, (HRM). This term implied geographical distribution, with an endemic focus in the Hagerman Valley area of southern Idaho. Because of continued spread of the organism, the Technical Procedures Committee of the Fish Health Section of the American Fisheries Society (Denver Workshop 1974) proposed the disease be given the more descriptive term of Enteric Redmouth Disease, (ERM).

Morphology and Staining

RM Bacterium is a straight or very slightly curved rod which averages 2-3 μ in length and 1 μ in width. In the 1950s the organism was thought to be lophotrichously flagellated (Klontz 1959, Rucker 1958). However, subsequent studies in the 1960s showed the organism to be Gram negative and peritrichously flagellated. Flagellation of the bacterium has been demonstrated by both flagellar stains (Lefison method) and electron photo-micrography. Occasional filamentous forms have been noted in older cultures grown for 48 hours at 25 C on trypticase soy agar (Ross et al. 1966).

Cultural Characteristics

Typical colonies on Trypticase Soy Agar or Tryptic Soy Agar are smooth, circular, and slightly raised with entire edges. They are not flourescent under ultraviolet light but appear slightly iridescent when examined by reflected light. Growth of the colonies is butyrous and non-pigmented. No color is imparted to the surrounding medium (Ross et al. 1966).

Colorless colonies appear on MacConkey Agar and Salmonella-Shigella Agar within 48 hours (22 C). Initially the growth on Bismuth Sulfite Agar is scanty and green, but after 3 to 7 days at 22 C the colonies turn olive green to black. RM Bacterium produced pink colonies when plated on Brilliant Green Agar at 22 C (Ross et al. 1966). McDaniel (1972) was unable to grow RM Bacterium (Willow Beach strain) on Brilliant Green Agar. However, he did get growth on Sellers Differential Agar, Salmonella-Shigella Agar, Devine EMB Agar without lactose, and Endo Agar.

The recent development of Rimler-Shotts (RS) medium has facilitated identification of various members of the family Enterobacteriaceae, including the RM Bacterium. On RS medium, colonies of the RM Bacterium appear yellow (Shotts, and Rimler 1973).

Biochemical Characteristics

Several biochemical properties of the RM Bacterium have been described independently by Ross et al. (1966), Busch (1973), and Wobeser (1973). Among the results are some differences in opinion (Table 1). Ross et al. and Wobeser reported negative hydrogen sulfide production, while Busch routinely detected lead sulfide in lead acetate media following 7 days incubation. Variability was also reported with the lysine decarboxylase and arginine dihydrolase results. In spite of the few inconsistencies, homogeniety among the various isolates is notable, especially with respect to carbohydrate fermentations.

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Table 1. Biochemical Reactions of RM Bacterium.

Test or Substrate	Ross et al (21) 37 C		Ross et al (21) 22 C		Busch (6) 25 C		Wobeser (27) 48 hr 23 C	
	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)
Hydrogen sulfide	0	14	0	14	44[a]	0	0	1
Urease	0	14	0	14			0	1
Urea Utilization					0	44		
Indol	0	14					0	1
Methyl Red	14	0	14	0	41	3		
Voges-Proskauer	0	14	0	14	0	44		
Neish test	0	14	0	14				
Citrate (Simmons')	0	14	13	1	44	0		
KCN	6	8	13	1				
Motility	6	8	14	0			1	0
Gelatin			14	0	44	0		
Lysine decarboxylase	13	1	14	0	39	5		
Arginine dihydrolase	2	12	0	14	26	18		
Ornithine decarboxylase	14	0	14	0	44	0		
Phenylalanine deaminase	0	14	0	14				
Glucose	14	0	14	0	44	0	1	0
Gas from Glucose	0	14	0	14				
Lactose	0	14	4	10	0	44[a]	0	1
Sucrose	0	14	0	14	0	44[a]	0	1
Mannitol	14	0	14	0	44	0		
Dulcitol	0	14	0	14				
Salicin	0	14	0	14	0	44[a]		
Adonitol	0	14	0	14				
Inositol	0	14	0	14				
Sorbitol	1	13	0	14	0	44[a]	0	1
Arabinose	1	13	0	14			1	0
Raffinose	2	12	0	14	0	44[a]	0	1
Rhamnose	0	14	0	14			0	1
Malonate	0	14	0	14				
Mucate	0	14	0	14				
Citrate (Christensen's)	5	9	14	0				
Kligler's Iron					B[b]	B&S[a]		
Triple Sugar Iron					B&S[b]	B&S[a]		
Hugh-Leifson					44	fermentative		
Catalase Production					44	0		
Starch Utilization					0	44		
Citrate	0	14	14	10				
D-Tartrate	0	14	0	14				
Jordan's Tartrate	14	0	14	0				
Sodium acetate	0	14	0	14				
Ammonium salt glucose	0	14	14	0				
Sodium alginate								
liquefaction	0	14	0	14				
utilization	0	14	0	14				
Corn oil	0	14	13	1				
Triacetin	0	14	0	14				
Tributyrin	0	14	0	14				
Pectate	0	14	0	14				
Maltose	14	0	14	0			1	0
Xylose	0	14	0	14				
Trehalose	14	0	14	0				
Cellobiose	1	13	0	14				
Glycerol	6	8	14	0	44	0		
Alpha methyl glucoside	0	14	0	14				
Erythritol	0	14	0	14				
Esculin	0	14	0	14				
Beta galactosidase	14	0	14	0				
Phenyl propionic acid	0	14	0	14				
Pigment	0	14	0	14			0	1
Cytochrome Oxidase	0	14	0	14	0	44	0	1
Nitrite	14	0	14	0	44	0	1	0

[a]-7 day incubation
[b]-24 to 48 hour incubation

B - Basic
S - Sulfide

Antigenic Characteristics

Forty-four isolates of RM Bacterium were examined by Busch (1973) and found to be biochemically and antigenically homogenous. Ross et al. (1966) noted antigenic cross reactivity with *Arizona 0* group as shown in Table 2.

Identification and the production of immunizing agents has been simplified by the homogeniety that exists with the RM Bacterium. A modified passive hemagglutination test has been developed to screen sera from populations of salmonids for the presence of agglutinations against the RM Bacterium (Busch and Lingg 1975).

Table 2. Antigenic relationship between the 0 antigens of the RM and Arizona Bacteria.

0 Antigen Suspensions	Unabsorbed	Absorbed by Arizona 26	Unabsorbed	Absorbed by RM
Arizona 26	640	0	640	0
RM	640	160	160	0

Based upon antigenic similarities, it has been suggested that there is a distant phylogenetic relationship among RM Bacterium, *Aeromonas liquefaciens*, and *Aeromonas salmonicida*. No cross-reactivity with *Pseudomonas fluorescens* or *Vibrio anguillarum* was noted (Hansen 1975).

Isolation Techniques

During an acute epizootic of ERM, RM Bacterium has been readily isolated from the kidney, liver, spleen, and gastrointestinal tract using classical methods of agar plating. However, when convalescent fish are sampled bacteriologically, the routine streaking method often gives negative results, especially if only the kidney and spleen are sampled. It has been reported (Anderson and Ross 1972) that during the convalescent phase of the disease, RM Bacterium could be isolated only from the lower intestine.

Trypticase Soy Agar (TSA) growth generally occurs within 24 hours at 25 C. Subcultures inoculated onto TSA slants have been stored at 5 C for up to 60 days without reculturing. More permanent storage of the organism has been accomplished by sealing wet packed cells in glass vials at -70 C (Busch 1973). Lyophilization of the organisms in rubber-stoppered glass vials with subsequent storage at -27 C has also been shown to be effective in maintaining its long term viability (Ross and Klontz 1965). RM isolates were shown to maintain their virulence following lyophilization in a sodium glutamate-gum tragacanth stabilizer with subsequent storage at 4 C (Busch 1973).

EPIDEMIOLOGY

Reservoir

Present evidence suggests that the organism is not ubiquitous in nature, since spread of the disease is associated with the movement of infected fish. Carrier fish and possibly other aquatic organisms in headwaters which supply hatcheries are suspected to be a significant RM Bacterium reservoir. Carrier fish in the raceways themselves are considered to be the major source of infection. The intestinal lining of carrier fish has been shown to harbor large numbers of the pathogen. These fish shed fecal casts containing RM Bacterium when stressed, and are thought to release organisms on a cyclic basis, even without being stressed. Fish with a clinical history of ERM are capable of continuously shedding the pathogen with infected feces, and serve as a source of recurrent infection to themselves and other susceptible populations for long periods of time on a regular cyclic basis. (Busch 1973).

Susceptible Species

Unpublished reports have indicated that the host range of the disease includes all salmonids and certain other species; however, rainbow trout are most commonly affected with ERM disease. The organism has been isolated from natural infections in rainbow trout (*Salmo gairdneri*), steelhead trout (*Salmo gairdneri*), cutthroat trout (*Salmo clarki*), sockeye salmon (*Oncorhynchus nerka*), fall and spring chinook salmon (*Oncorhynchus tshawytscha*) (Busch 1973, Holt and Conrad 1970) and coho salmon (*Oncorhynchus kisutch*) (McDaniel 1971). The disease has been transmitted to Atlantic salmon (*Salmo salar*) by injection and by the addition of bacterial suspensions to the water (Bullock and Snieszko 1975). Margolis, in an unpublished observation cited by Rucker (1958) described an RM-like organism from diseased suckers in Canada.

Range

In 1966 the geographical range of the disease was reported (Ross et al. 1966) to include Idaho, Colorado, Nevada, California, and Arizona. By 1971 reports (McDaniel 1971) indicated the disease had further spread to Alaska, Washington, Oregon, Utah, and Wyoming. In 1973 the first documented case (Wobeser 1973) occured in Saskatchewan, Canada. Sporadic outbreaks have occured since in Nebraska (McElwain 1975), Missouri (Camenisch 1975), Ohio (Bullock and Snieszko 1975), and as far east as Tennessee (McCraren and Warren 1973). Present evidence suggests these expansions of the ERM geographical range are attributable to movement of infected fish (Bullock and Snieszko 1975, Wobeser 1973).

Occurrence

ERM outbreaks generally occur only when fish are exposed to large numbers of the RM Bacterium (Ross et al. 1966). Large scale epizootics may occur if chronically infected fish are stressed during handling, exposed to low dissolved oxygen or other poor environmental conditions (Bullock and Sneiszko 1975). Weighing or moving apparently healthy fish is known to have been succeeded by an epizootic (Rucker 1966).

The incubation period was reported to vary from 5 to 19 days depending on the virulence of the organism (Bullock and Snieszko 1975, Busch 1973, Rucker 1966), as well as upon environmental conditions (Bullock and Sneiszko 1975).

The size of rainbow trout commonly affected was initially reported as 7.5 cm in length. The naturally occuring disease was thought to be less severe and more chronic in fish 12.5 cm and longer (Rucker 1966).

The severity of the infection was shown to decrease at water temperatures below 10 C (Rucker 1966). The nutritional status is another important factor which affects the susceptibility of the fish to ERM. Overly fat or debilitated fish are thought to be more susceptible to severe epizootics (Rucker 1966).

Occurrence may be cyclic after the initial infection in ponds. On the basis of pathogen recovery, gross pathological changes, and mortality rates, it appears that a regular 36 to 40 day cycle of RM Bacterium being shed from the intestinal wall of convalescent fish does occur. Under laboratory conditions this shedding is thought to precede the reappearance of gross pathological change and mortality by 3 to 5 days. This cyclical shedding could precipitate throughout the year. Cyclic shedding is thought to be altered by seasonal variation in water temperature, loading factors, handling, natural resistance and immunity of the population (Busch 1973).

There have been no reports suggesting a seasonalit pattern for epizootics. However, McDaniel (1971 showed yearly cyclical mortality patterns in a larg untreated hatchery population chronically infecte with RM Bacterium.

A 2.0-2.7 percent natural carrier incidence wa recorded among naturally infected salmoni populations in southern Idaho. One recently infecte hatchery population had a carrier incidence of 6.: percent (Busch and Lingg 1975). These percentage were based upon the passive hemagglutination test fo the detection of RM carrier fish, although nothing ha been documented which correlates the presence o agglutinating antibodies to actual harboring of th pathogen within the host.

Transmission

Water has been shown to be an effective vehicl for transmission of ERM disease. Dead fish were placec in the head compartment to a trough containing 5(healthy 7.5 cm rainbow trout (15 C). These previously unexposed fish suffered a 52 percent mortality from 5 to 19 days post-exposure. In a second experiment, a broth culture of RM Bacterium (approximately 50(cells/ml) was administered by continuous drip for 4 days into a trough containing 50 10 cm fish in 15 C water. Mortalities attributable to ERM disease occurred from 5 to 18 days post exposure (Rucker 1966).

The in vitro survival of RM Bacterium was tested on sterile nylon netting and in filtered sterilized water. Pieces of the netting were soaked for 15 min in a sterile water suspension containing 540 RM Bacterium cells/ml. The pieces of netting were incubated at 21 C in sterile petri dishes and allowed to dry for varying time periods. Addition of nutrient broth after one hour of drying revealed growth. Addition of nutrient broth after 24 hours produced no growth of RM Bacterium. Drops of the sterile water and RM Bacterium suspension placed in sterile petri dishes were shown to contain viable organisms for up to 48 hours. The nylon netting required 24 hours to dry in a petri dish, and a 2 ml drop of the suspension was dry after 6 to 7 days (McDaniel 1972). These results indicate the possibiltiy of cross-infection of ponds by nets.

Preliminary tests were conducted on the survival of the organism in natural water and natural water plus raceway solid waste. Information from these preliminary trials indicates that the RM Bacterium may be well adapted to life as an aquatic saprophyte (McDaniel 1972).

PATHOLOGY

Gross Lesions

The first description of the gross pathological changes occurring subsequent to RM Bacterium infection were reported by Rucker (1966). He described affected fish as typically being sluggish, dark in color, and having the characteristic reddening in the mouth, on the opercula, about the isthmus, and on the base of the fins. This reddening is reportedly due to subcutaneous hemorrhage (Busch 1973). Small, open hemorrhagic lesions on the anterior lateral aspect of many affected fish were also reported. An exophthalmic condition commonly occurs which generally progresses from unilateral to bilateral exophthalmia, with occasional rupture of the eye from its supporting tissue (Busch 1973). Hemorrhages in the periocular region have also been reported (Hansen 1975).

The internal gross pathological changes include petechial hemorrhages in the liver, muscle, adipose tissue, swim bladder, and coelomic mesenteries. Splenomegaly along with hemorrhagic reddening of the gonadal tissue and the distal ends of the pyloric ceca also occurs. The lower intestinal tract is inflamed, flaccid, and filled with a yellow mucoid material from which the organism can be readily recovered (Busch 1973).

The degree of splenic hypertrophy has been quantitatively reported by using a spleen/total body weight (S/T) ratio. In this study, one pond contained both asymptomatic and moribund ERM diseased fish. A second pond contained control fish which were never exposed to the RM Bacterium. The average S/T ratio of the moribund RM Bacterium infected fish was 3.30 times that of the asymptomatic group and 3.37 times that of the unexposed group (Klontz 1959).

Microscopic Lesions

Rucker noted the histopathological changes of ERM disease to be characteristic of those in any acute bacteremic infection of trout. He isolated RM Bacterium from virtually all tissues, especially the highly vascular tissues such as the kidney, spleen, liver, heart, and gill. All the organs exhibited the microscopic changes consistent with a marked inflammatory response. Phagocytosis of the organism by macrophages and large lymphocytes was noted throughout most of the tissues, especially in the kidney (Rucker 1966).

Necrotic destruction of the hematopoietic tissue within the spleen and kidney sinusoids along with a decrease in size and number of pigment cells and swelling and vacuolation of reticuloendothelial (RE) cells also occurred. A total loss of the normal lymphoid follicular structure within the spleen along with erythrocytic congestion of the splenic sinusoids and RE cell hypertrophy has also been reported (Wobeser 1973).

There was little to no vacuolation of the hepatocytes reported in these ERM affected fish. RE cells lining the hepatic sinusoids were found to be swollen and prominent with vesicular nuclei. There was marked accumulation of mononuclear cells present in the periportal areas of the liver. Retrobulbar edema and panophthalmitis along with intraocular hemorrhage and inflammatory cell infiltration also were noted. Wobeser (1973) reported the inflammatory cells within the eye to be a mixture of polymorphonuclear leukocytes and macrophage-like cells.

Differential cell counts of RM infected fish showed that moribund ERM disease fish had one-third the lymphocytes of the asymptomatic and unexposed groups. There was also a general increase in relative numbers of lymphoid hemoblasts on all the imprint smears from fish in the moribund group as compared to the other two groups. This decrease in the relative numbers of lymphoid hemoblasts, and an increase in relative numbers of lymphocytes in the spleen were associated with antibody production (Klontz 1959).

Table 3 shows the relative difference of blood cell types among the 3 groups of fish. The moribund and asymptomatic groups came from the same pond during an ERM epizootic. The unexposed group came from a pond which had never experienced ERM mortalities.

Pathogenesis

The sequential pathological changes occurring during the course of an RM Bacterium infection have not been described in detail. The specific cause of death among fish suffering from ERM disease has not yet been reported.

IMMUNOLOGY

Naturally Acquired Immunity

Agglutinating and non-agglutinating antibody studies were conducted to determine the possibility of naturally acquired immunity in rainbow trout against RM Bacterium. The tested fish had not been parenterally immunized but had been exposed to RM Bacterium in ponds. The antibody titers against RM Bacterium found in these fish indicate some naturally acquired immunity occurs (Klontz 1957). These results were not equated to protection against the organism.

Table 3: Differential counts of cells from kidney, spleen, liver and heart blood of rainbow trout affected with ERM.

Fish Group	Cell Count Samples[a] Kidney					Spleen					Liver					Heart Blood				
	Ma	PC	He	Ly	Gr[a]	Ma	PC	He	Ly	Gr[a]	Ma	PC	He	Ly	Gr[a]	Ma	PC	He	Ly	Gr[a]
I	14	7	47	28	4	28	3	19	23	7	50	0	15	16	19	44	2	30	22	2
II	2	8	32	34	24	2	0	3	82	12	5	1	5	72	16	5	1	7	75	13
III	5	2	38	34	21	4	0	4	84	8	4	0	3	87	7	2	1	5	90	2

NOTE: These numbers represent the average cell counts for each type of cell in a sample of 7 fish for each of the three groups.

SOURCE: Klontz 1959

Fish Group, I – moribund; II – asymptomatic; III – unexposed

Ma = macrophage; PC = plasma cell; He = lymphoid hemoblast;
Ly = lymphocyte; Gr = granulocyte

Artificially Acquired Immunity

Studies to develop a means of artificially immunizing rainbow trout against ERM disease began in 1962. An orally administered phenol-killed bacterin afforded good protection under laboratory conditions. Orally immunized fish had a 90 percent survival rate whereas controls had a 20 percent survival rate when challenged by inoculation of 1-40 LD_{90} of virulent RM organisms (Ross and Klontz 1965). (See Table 4.)

During the 1968 field trials at the Willow Beach National Fish Hatchery the presence of RM antibodies was demonstrated by using the fluorescent antibody technique following the feeding of this same oral bacterin. The level of protection against a natural ERM epizootic at this hatchery however was shown to be unsatisfactory (McDaniel 1968).

A study was conducted comparing the effectiveness of four different oral bacterin preparations in inducing protection against ERM. Over a period of 6 weeks each fish received an estimated 1,500 µg of the organism. The fish were challenged by a subcutaneous injection of the homologous, virulent RM bacteria in increasing concentrations. The LD_{50} was calculated for each of the four groups. Upon challenge, all the bacterin-fed groups had a lower mortality pattern than the controls. The chloroform-killed bacterin engendered greater protection than the other preparations. The number of RM Bacterium required for the LD_{50} among the four orally innumized groups is presented in Table 5.

Anderson and Ross (1972) suggested that the immune response could be overwhelmed by large numbers of virulent bacteria, and in such a situation even the most efficient method of immunization would probably be of little value.

A similar study was conducted which compared protection afforded rainbow trout by the oral route versus the parenteral route of RM bacterin administration. In this study a 3 percent chloroform-killed bacterin was utilized.

Anderson and Nelson (1974) reported that protection against the ERM pathogen was demonstrable in both groups of trout. However, the inoculated fish had higher levels of protection and retained this protection longer than did the orally immunized fish. The orally immunized group had similar protection against an RM Bacterium challenge as did the unimmunized controls at 8 and 13 weeks post immunization (Anderson and Nelson 1974).

Table 4. Summary of response of immunized and control rainbow trout to challenge doses of virulent redmouth organisms at several time intervals.

	Survivors			
Days	Immune	Control	Dosage	
70	9/10	1/10	1 LD_{90}	
98	10/10	4/10	1 LD_{90}	
304	9/10	1/10	1 LD_{90}	
339	9/9	4/10	5 LD_{90}	– previous challenge: 304 days
	10/10	1/10	1 LD_{90}	
360	7/10[a]	2/10[b]	1 LD_{90}	
		1/10	1 LD_{90}	
	11/16	0/10	40 LD_{90}	
408	9/9	1/10	10 LD_{90}	– previous challenge: 304 and 339 days
Total:	45/50 (90%)	12/60 (20%)	1 LD_{90}	
	9/9 (100%)	1/10 (20%)	10 LD_{90}	
	11/16 (69%)	0/10 (0%)	40 LD_{90}	

SOURCE: Ross and Klontz 1965.

a These fish were fed a mixture of redmouth and *A. salmonicida* bacterin.
b These fish were fed *A. salmonicida* bacterin.

DIAGNOSIS

Clinical Signs

ERM disease exhibits similar clinical signs to most other acute bacteremias; therefore, a definitive diagnosis of ERM cannot be made on the basis of clinical signs alone. Two other fish pathogens cause syndromes in fish that could easily be confused with ERM, namely the *Aeromonas liquefaciens* complex and *Aeromonas salmonicida*. The use of specific biochemical tests, flourescent antibody technique, or specific RM antisera is necessary before a definitive diagnosis can be made.

Fish in the acute phase of ERM are typically sluggish, have no interest in feeding, and exhibit the classical reddening in and around the mouth, on the opercula and isthmus, at the base of the fins, and about the vent. Fish in the chronic phase of ERM often appear dark in color. Often the abdomen is distended and exophthalmia occurs. The latter condition may either be unilateral or bilateral (Busch 1973, Hansen 1975, Rucker 1966, Wobeser 1973).

Laboratory findings

Differential leukocyte counts from circulating blood of fish clinically ill with ERM revealed a high percentage of macrophages (Klontz 1959). ERM affected fish exhibited hematocrit readings averaging 23 percent and total protein values 2.8 g/100 ml. Normal fish tested in the same study had an average hematocrit of 46.1 and a total protein of 4.7 g/100 ml (Wobeser 1973).

Immunofluoresence recently has been applied as a means of obtaining definitive diagnosis of ERM. The indirect fluorescent antibody technique for RM Bacterium effectively detected the organism in washed cultures, broth cultures, and in kidney imprint smears from experimentally infected fish. Because of the high specificity and sensitivity of the technique, the

pathogen can be detected without incubation to increase bacterial concentration.

Immunofluorescence can be advantageous over standard microbiologic methods when the sample is contaminated, the organisms are nonviable, or when rapid diagnosis is desired. The addition of a rhodamine-labelled counterstain was shown to enhance the efficacy of the technique by staining fish cells orange to contrast with the green halo around the RM Bacterium (Johnson et al. 1974).

The use of specific RM antiserum has been shown to be an effective, rapid method of obtaining a definitive diagnosis. (Bullock and Snieszko 1975).

Another laboratory test has been developed recently for detection of RM antibodies in acute and chronically ill or post-epizootic fish. The inert particle agglutination test appears to be ideally suited for use in large scale serological surveillance programs. It is reported to be rapid, reproducible, sensitive, and specific. Sensitized particle suspensions were made in advance and reportedly were stable at refrigerator temperatures for up to a year.

A microhematocrit centrifuge is the only piece of equipment required for the inert particle agglutination test. The serum volume required for the test was small (24 µ 1), and obtained by the microhematocrit technique. Once a serum sample is prepared the actual test takes less than 10 minutes to complete (Hansen 1975).

Table 5. Numbers of redmouth bacteria required for LD_{50}.

Control fish	8.5×10^5
Test fish	
Vaccines prepared by:	
0.5% phenol	3.0×10^7
3.0% chloroform	9.0×10^9 (estimate)
Sonification, 1.0% formalin	8.2×10^6
3% phenol, dialysis	1.8×10^8

SOURCE: Anderson and Ross 1972.

Differential Diagnosis

It is difficult to differentiate an acute ERM infection from that of any other acute systemic bacterial disease based upon clinical signs. Acute bacteremias in trout which are caused by a short, Gram negative rod and exhibit similar clinical signs pathological changes are *Aeromonas salmonicida*, th causative agent of furunculosis, and *Aeromon liquefaciens* complex, which causes diseases common referred to as redmouth, and bacterial hemorrhag septicemia.

If the organism is motile and cytochrome oxidas negative, then a tentative diagnosis of ERM could b made. To confirm the diagnosis, specific antiser passive hemagglutination test, fluorescent antibod technique (Johnson et al. 1974), or the inert particl agglutination test (Hansen 1975) could be made.

The following flow chart (Fig. 1) could be used t aid in differentiation of the Gram negative bacteri commonly associated with fish diseases (Shotts an Bullock 1975).

TREATMENT AND CONTROL

Various antibacterial agents incorporated into the feed have been used as chemotherapeutics for ERM disease. Sulfamerazine and oxytetracycline (Terramycin^R) have been used most often to control epizootics of this disease. Sulfamerazine at 9 g/100 lb of fish (200 mg per kg body weight) per day for 5 days followed by 3 days of chloromycetin (Chloramphencol^R) or oxytetracycline (Terramycin^R) at 2.5 g/100 lb of fish (50 mg per kg body weight) per day is one method of treatment using individual administration of antibacterial agents (Ross et al. 1966). Variation of these treatments has been reported successful as well. In one documented ERM outbreak, the use of oxytetracycline at 2500 g/ton of feed was shown to be less effective than sulfamerazine at 200

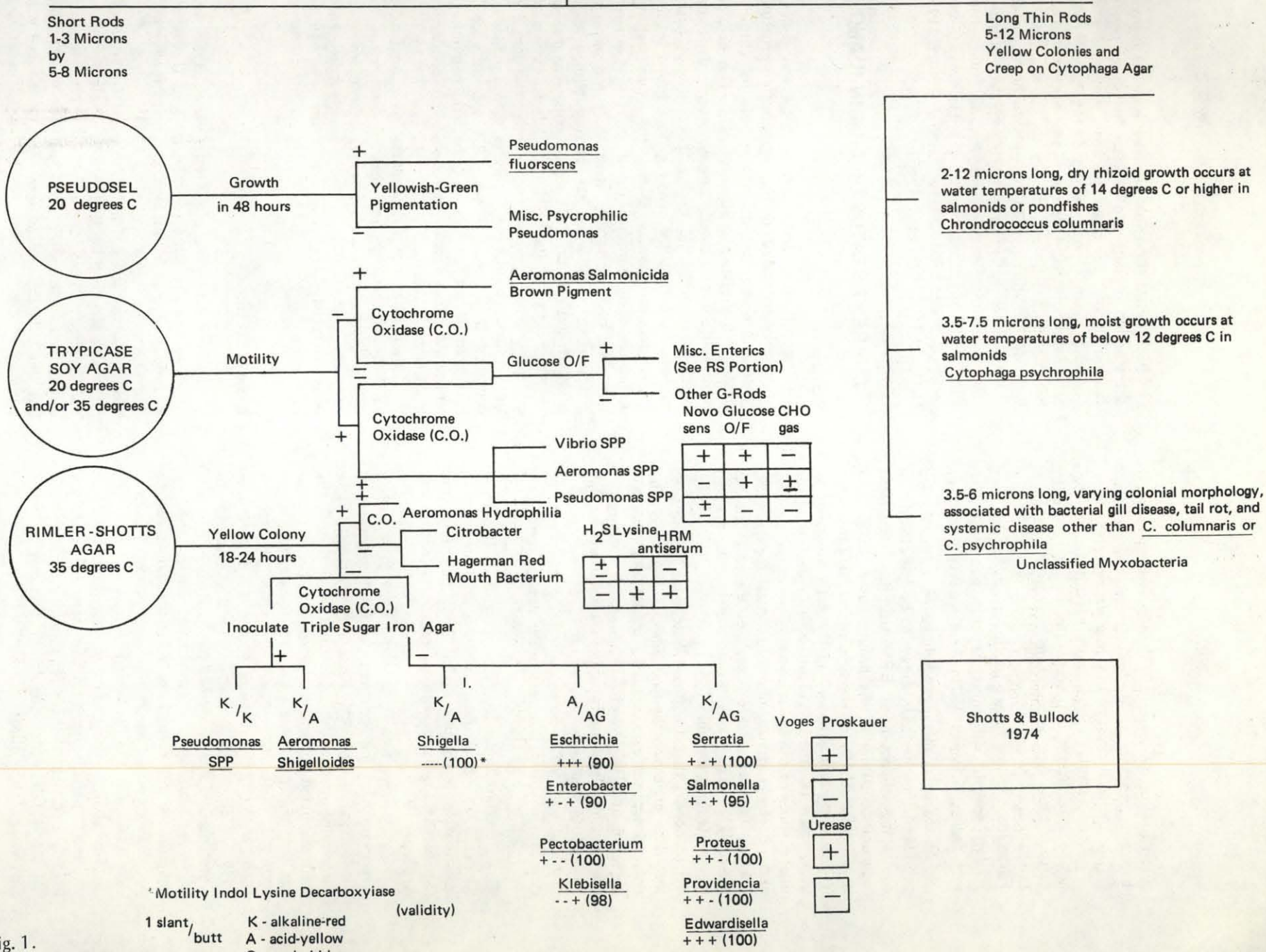
Short Rods
1-3 Microns
by
5-8 Microns
Long Thin Rods
5-12 Microns
Yellow Colonies and
Creep on Cytophaga Agar
PSEUDOSEL
20 degrees C
Growth
in 48 hours
Yellowish-Green
Pigmentation
Pseudomonas
fluorscens
Misc. Psycrophilic
Pseudomonas
TRYPICASE
SOY AGAR
20 degrees C
and/or 35 degrees C
Motility
Aeromonas Salmonicida
Brown Pigment
Cytochrome
Oxidase (C.O.)
Glucose O/F
Misc. Enterics
(See RS Portion)
Other G-Rods
Novo Glucose CHO
sens O/F gas
Cytochrome
Oxidase (C.O.)
Vibrio SPP
Aeromonas SPP
Pseudomonas SPP
RIMLER-SHOTTS
AGAR
35 degrees C
Yellow Colony
18-24 hours
C.O.
Aeromonas Hydrophilia
Citrobacter
Hagerman Red
Mouth Bacterium
H2S Lysine HRM
antiserum
Cytochrome
Oxidase (C.O.)
Inoculate Triple Sugar Iron Agar
K/K
K/A
K/A
A/AG
K/AG
Pseudomonas
SPP
Aeromonas
Shigelloides
Shigella
-----(100)*
Eschrichia
+++ (90)
Enterobacter
+-+ (90)
Pectobacterium
+--(100)
Klebisella
--+ (98)
Serratia
+-+ (100)
Salmonella
+-+ (95)
Proteus
++-(100)
Providencia
++-(100)
Edwardisella
+++ (100)
Voges Proskauer
Urease
2-12 microns long, dry rhizoid growth occurs at water temperatures of 14 degrees C or higher in salmonids or pondfishes
Chrondrococcus columnaris
3.5-7.5 microns long, moist growth occurs at water temperatures of below 12 degrees C in salmonids
Cytophaga psychrophila
3.5-6 microns long, varying colonial morphology, associated with bacterial gill disease, tail rot, and systemic disease other than C. columnaris or C. psychrophila
Unclassified Myxobacteria
Shotts & Bullock
1974
*Motility Indol Lysine Decarboxyiase
(validity)
1 slant/butt
K - alkaline-red
A - acid-yellow
G - gas-bubbles

Fig. 1.

g/ton of feed (Wobeser 1973). Although this suggests bacterial resistance to the oxytetracycline, no reason for the ineffectiveness of this drug was reported.

The simultaneous administration of two antibacterial agents has been effective in controlling certain ERM epizootics. One successful treatment program used 2 g of sulfamerazine and 125 g of Furazoldine (Furox^{R}) per 100 lb of fish per day for a 15-day period. Variations of this treatment have reportedly (McDaniel 1971) been successful as well.

Antibacterial therapy administered for prolonged periods of time has been shown to be less effective in controlling epizootics of ERM than have treatment regimens consisting of the proper dosage for 5 to 7 days (McDaniel 1971). The time of initiating treatment is also important. Therapy in the early stages of an ERM epizootic is effective in terms of decreasing the total number of fish dying and in terms of the quantity of antibacterial agent required to control the outbreak. Therapy initiated after the peak of the epizootic has passed is, in many cases, no more efficacious than no therapy (Klontz unpubl.).

Iodophors (Betadine^{R} and Wescodyne^{R}) have been shown to effectively destroy 5 x 10^{6} RM cells after only 15 seconds exposure in a recent laboratory test (15 ppm at pH 7.0 and 8.0). Although nothing has been documented concerning egg transmission of ERM, it was suggested (Ross and Smith 1972) that exposing salmonid eggs to 25 ppm iodine for 5 min would be effective in killing RM cells under natural conditions.

Control of ERM disease by oral immunization has shown promise under laboratory conditions (Ross and Klontz 1965). Subsequent hatchery trials resulted in questionable protection against both parenteral and natural challenge with virulent organisms (Klontz and Anderson 1970).

Management techniques could have an important role in reducing the occurrence of ERM epizootics by elimination of the RM Bacterium reservoirs. Since chronically infected fish are carriers which periodically shed the pathogen into the water, and because water has been shown to be a vehicle for transmission of the organisms, these fish represent a reservoir of infection which should be eliminated (Busch 1973, McDaniel 1972, Rucker 1966). This necessitates removal of symptomatic ERM fish from the hatchery waters and incoming waterways. Transported carrier fish or symptomatic fish within the hatchery should not be placed in receways which flow into ponds containing ERM disease-free fish (Wobeser 1973).

The passive hemagglutination test (Busch 1973) or the inert particle agglutination technique (Hansen 1975) are possible methods for detecting asymptomatic RM carrier fish or fish populations that may be in the early stages of the disease.

Because RM Bacterium can survive on netting for short period of time and in water for longer periods caution should be taken to avoid transferring th organism from pond to pond (McDaniel 1972) Sanitization of nets, waders and other equipment coul be accomplished by an iodophor such as Betadine^{R} o Wescodyne^{R} (Ross and Smith 1972).

Avoidance of stress due to improper nutrition adverse environmental conditions, and handling ha also been suggested as a possible managemen technique for ERM disease control (Busch 1973 McDaniel 1971, Ross et al. 1966).

CURRENT STATUS OF ERM IN IDAHO

Background

The following description of ERM was collated from opinions stated during a 3-hour unstructured conference with many Idaho commercial fish culturists and fish biologists on 30 July 1975. The opinions rendered were based largely upon day-to-day experiences with ERM during the course of raising rainbow trout from the eyed-egg stage to market-size.

It became obvious during the course of the discussion that individual fish farmers saw different forms of ERM. In many cases there was apparent disagreement with what has been published about ERM. In this respect, the disagreements were resolved by recognizing that the ERM in the laboratory was not quite the same as that at hatcheries or fish farms. In some instances during the discussion, several individuals conceded that some of their ERM epizootics could have been in fact furunculosis or bacterial hemorrhagic septicemia. Both occur sporadically on commerical trout farms in Idaho.

This is offered as a contribution to understanding ERM better, remembering that in most cases the following statements are subjective observations which in many instances have not been technologically substantiated. We support them all without reservation.

Name of the Disease

Currently there are many terms vocalized denoting ERM. Among the more common are Red Throat and Red Mouth or Red Mouth Disease. The term Hagerman Red Mouth has been applied to ERM in areas of the country outside Idaho. The Idaho trout farmers would rather that this term be dropped completely because of the implied stigma associated with it. Another colloquial term denoting ERM has been Pink Throat, but this term has seldom been heard in the past 5 to 10 years.

Definition

The current consensus of opinion about ERM is that it is an acute to chronic systemic bacterial disease of rainbow trout with other species of salmonids being affected irregularly. The disease is characterized by a slow, daily increase in mortality in fish from 4/lb to 1/lb with dead fish exhibiting frayed fins, red blotches on the body, protruding eyeballs, fluid in the body cavity, and a yellowish gut fluid.

Etiology

The cause of ERM is a Gram negative rod occurring in singles, pairs, and short chains in all tissues of the affected fish. The medium of choice for isolation is trypticase soy agar with incubation at 20-29° C for 24 to 28 hours. Although the published and accepted name of the causative organism is RM Bacterium, most fish farmers having had encounters with ERM call the organism the Red Mouth Bug.

Epidemiology

Geographical Distribution: ERM in the 1950's was endemic to the Hagerman Valley in the region of the emergence of the Snake River Plain aquifer. This region has been known as Thousand Springs. In the past 10 to 15 years the causative organism has been transported via live, asymptomatic carrier fish throughout the United States and Canada. In a few places the introduced RM Bacterium has become endemic and caused repeated epizootics.

Transmitting Agents: The reservoir of the RM Bacterium is considered to be resident fish in the fish farm water supplies. This is an established conclusion, as fish chronically ill with ERM have been removed from several of the springs at various times. Snails, crayfish and sculpins, all resident in the majority of the Thousand Springs, are also considered to harbor RM Bacterium. However, there is no substantiating evidence to support this contention. RM Bacterium is transmitted from fish to fish via the water. There is no evidence suggesting that there might be an egg transmission. Infection apparently occurs via the gastrointestinal tract or the respiratory epithelium, more likely the former than the latter.

Susceptible Hosts: ERM is primarily an infectious disease of rainbow trout. The albino or Idaho Golden trout seems to be less susceptible to ERM than the pigmented rainbow trout. Outbreaks of ERM have occurred spontaneously in coho salmon and cutthroat trout. It is not known if any other salmonids are affected by ERM since others are not reared in the endemic areas.

Factors Influencing Susceptibility: It is generally agreed that ERM outbreaks are cyclic in pattern and occurence. However, cycles are more related to a particular trout farm than to the geographic area or season of the year. As some trout farmers put it, "There are good years and there are bad years. You just never know until it's too late."

It is also generally agreed that outbreaks can be precipitated by "working" (a term denoting handling fish in any fashion) fish. As a rule the outbreak follows the handling stress by 3 to 5 days.

When ERM first became a noticed problem in the raising of trout, the outbreaks occurred most frequently in the 6 inch to 9 inch fish (14/lb to 5/lb). In the past 2 to 3 years the 9 inch to 12 inch fish (5/lb to 1/lb) are most affected. Thus, by virtue of the larger fish being affected, economic losses have increased significantly.

Clinical Signs

There are obviously two clinical manifestations of ERM. One is called the "red" form and the other the "dark" form. Both may or may not be seen during any given outbreak.

The dark form can manifest itself one of two ways. One, by the "hanger-outer" fish which are seen prior to an acute or sub-acute epizootic. These fish do not feed, and isolate themselves apart from the main school of fish in the pond. Other than being noticeably dark and depressed, they appear quite healthy. Second is the appearance of "screen hanger" fish which are seen for some time following an acute outbreak. These fish are either swimming just in front of the tail screen or are pressed against it by the current. These fish are emaciated and bilaterally exophthalmic – or blind because both eyes are gone as a result of the exophthalmia. The question of whether the "hanger-outer" fish became "screen hanger" fish is still open to resolution.

Externally, the dark fish, especially the screen hangers, are very dark due to melanin dispersal, have frayed fins, are uni- or bilaterally exophthalmic (20-25 percent incidence), blind, slow moving with virtually no avoidance reaction to a net, emaciated, and occasionally have reddened vents. Internally, they have a pale, mottled liver, an empty gastrointestinal tract, virtually no visceral fat, and an enlarged spleen and kidney. There may or may not be petechial hemorrhages in the internal body wall and a moderate amount of yellowish abdominal fluid.

The red form occurs during the acute stage of the outbreaks. The overall general body coloration may be no different than unaffected fish or it may be slightly lighter. The body conformation of these fish is no

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different than the rest of the population. This is somewhat contrary to what is described, in that ERM affects those fish which are not in the best of physical condition.

Externally, the red fish have frayed fins with hemorrhaged bases, petechiation of the ventral body wall and oral cavity, abdominal distention, and an inflamed vent with a bloody mucoid discharge. There also may be the early signs of either uni- or bilateral exophthalmia.

Internally, red fish have an enlarged spleen and kidney, but not to the degree seen in dark fish. There are also petechiae throughout the viscera and visceral wall. The stomach may or may not have ingesta. The upper intestine often contains a yellow mucoid fluid which becomes reddened in the lower intestine near the vent.

Treatment

Current treatment methods consist of feeding either oxytetracycline or sulfamerazine, or both simultaneously, in the diet at the FDA levels for the prescribed time. One of the major concerns is that these regimens are not maintaining their effectiveness. Also, since near market-size fish are being affected more and more, it is difficult to treat these fish and stay within the withdrawal time guidelines.

Prevention

Several approaches to preventing outbreaks ERM have been tried. One has been to administ oxytetracycline for 3 days in succession a few da prior to the fish being worked. Two days prior being worked, the fish are fed nothing, there minimizing the stress effect on the intestinal trac the presumed site of the RM Bacterium in the carri fish.

Another method used successfully by or commercial trout farmer and at one National Fis Hatchery is to remove by electroshocking c chlorination all fish in the headwaters leading to th facility. In both instances there have been n detectable occurrences of ERM since these measure were taken. Since the 30 July conference, at least tw commercial trout farms have electroshocked thei water supplies and head ditches. In one of these several hundred pounds of fish were removed.

Management methods have also been used wit varying degrees of success. These consist of cleanin ponds more frequently, keeping pond loadings wel below maximum limits, sanitizing nets and brooms and disinfecting ponds following removal of the fish.

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