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Enteric Red Mouth Disease (Hagerman Strain)

R. A. BUSCH

ABSTRACT — Enteric red mouth (ERM) disease of salmonid fishes is reviewed in terms of description of the etiological agent, pathogenesis and diagnosis of the clinical condition, and epizootiological considerations pertinent to its effective control. Recent studies defining the asymptomatic carrier state of the disease are discussed. Enteric red mouth disease is shown to establish an asymptomatic carrier state infection in the lumen of the lower intestine in 50 to 75 percent of epizootic survivors 60 to 65 days postinfection. This chronic carrier state is maintained for more than 102 days in the experimental population. A cyclical intestinal shedding pattern develops with a periodicity of 36 to 40 days and serves to cause regular reinfection and mortality.

A serological presumptive screening test for ERM is described. The system is shown to be sensitive and specific and readily adapted to the rapid screening of large numbers of individual small volume sera for presumptive evidence of specific disease association at a minimum cost and without sacrifice of fish over 15 cm in length. Based upon serological screen field data, ERM is shown to consistently develop a 2.0 to 2.7 percent carrier incidence among populations following epizootic infection. The humoral immune response of rainbow trout to various antigenic preparations of the ERM bacterium is examined. Various soluble protein fractions are shown to be more immunogenic than lipopolysaccharide fractions. A particulate cell wall antigen and several other killed whole cell antigens resulted in high titers while a heat-killed whole-cell preparation is shown to be nonimmunogenic. Serum agglutinin titers declined at a monthly rate of 9.8 percent following induction and were detectable for more than 9 months. Evidence is given to support the theory that serum agglutinins in trout are IgM-like macroglobulins.

Enteric red mouth (ERM) disease was first recognized as a cause of mortalities in rainbow trout, *Salmo gairdneri*, in the late 1950's in the Hagerman Valley of Idaho. Subsequently, the disease was studied by Ross et al. (1966) and Rucker (1966). These workers were unable to give the etiological agent a more definitive taxonomic position, but based upon morphological, biochemical, and serological data, they placed it in the family Enterobacteriaceae.

EPIDEMIOLOGY

The ERM bacterium was originally referred to as the "RM bacterium." This terminology tended to confuse it with other bacterial pathogens that cause a similar clinical syndrome. McDaniel (1971) proposed the name Hagerman red mouth or HRM, describing its original range. In 1975 the Fish Health Section of the American Fisheries Society chose to remove this onus on the Hagerman Valley and called it enteric red mouth disease, indicating that the causative agent is one of the two major enteric pathogens of fish in fresh water: the other being *Edwardsiella tarda*. Enteric red mouth disease has also been referred to in the literature as red mouth disease, red vent disease, and bacterial hemorrhagic septicemia, but in many instances, authors were describing a clinical syndrome that was often of diverse or multiple etiology rather than the etiological agent itself.

In 1966, ERM was found to be endemic to the Hagerman Valley of Idaho as well as the States of California, Nevada, Arizona, and Colorado. When one studies the epidemiology of this geographically isolated disease and its dissemination, one often finds that it was introduced with subclinically infected stocks of fish acquired from an area where it is endemic. As an example, in the middle and late 1960's, the trout industry in the Hagerman Valley shipped large numbers of asymptomatically infected live fish to various western states for grow-out and fish-out operations. During that time the disease was widely disseminated, and in recent years economically important geographical range extensions and epizootics of ERM were documented to occur due to transporation of asymptomatically infected stocks into previously disease-free areas (Wobeser, 1973). By 1970, the originally recognized range of occurrence had expanded to include Alaska, Oregon, Utah, Washington, and Wyoming. More recently, it has been reported from British Columbia, Montana, Nebraska, Ohio, Saskatchewan, and Tennessee. The disease has since been reported from various other places outside of North America, particularly Italy, but these reports, to my knowledge, have not been serologically confirmed. It is significant to note that ERM still remains comparatively limited in its geographical distribution on

R. A. Busch is with the Fish Pathology Laboratory, School of Natural Resources, Humboldt State University, Arcata, CA 95521. Present address: Rangen Research Hatchery, Rt. 1, Hagerman, ID 83332.

a worldwide watershed basis and, therefore, demonstrates an excellent potential for preventative management and an eradication type of program in comparison to other more ubiquitous fish pathogens.

ECONOMIC EFFECTS

Some causative agents of fish diseases are ubiquitous in their occurence and even free living in certain instances, while other pathogens are fairly fastidious and geographically isolated, as is the case with ERM. This will have a definite practical bearing on how we are going to prevent, manage, treat, and handle stocks of fish infected with ERM and reduce the adverse economic impacts of the disease on the trout industry.

In the Hagerman Valley, commercial fish farms located along a 35-km stretch of the Snake River canyon produce about 80 percent of the commercial rainbow trout in the United States. These are private and highly competetive operations. It is difficult to get accurate figures, but this production amounts to over 9,000 metric tons (t) of rainbow trout a year. One hatchery that has just been put into operation has an annual production capacity of 2,250 t of trout based upon a flow of 9.20 m^3 per second of spring water from the aquifer of the Snake River plain.

Araji (1972) did an economic study on the impact of disease on the trout industry in the Hagerman Valley and estimated an annual loss of approximately $750,000 or roughly 10 percent of the total production costs. I have seen loss estimates as high as 35 percent of production costs and up to $2½ million annually depending upon the operation and management involved. It is interesting to note a recent study by Klontz and King (1975) on the exact nature of these losses. If you were to follow losses due to disease in egg, sac fry, swim-up and fingerling stages, and on up to marketable 15- to 30-cm fish, the total number of mortalities are greatest in the egg through swim-up fry stages and decrease as the fish get older. However, while the mortalities in marketable size fish represents only 14 percent of the total mortalities, it amounts to 76 percent of the costs in dollars. The mortality in the small fish in these hatcheries is mainly attributed to bacterial gill disease and other management-related conditions as well as infectious pancreatic necrosis, a presently untreatable viral disease. In comparison, losses in the marketable size fish are attributed to ERM and other treatable bacterial diseases. This concept is of primary importance in the consideration of the economics of disease prevention and treatment in these stocks.

CAUSATIVE ORGANISM

Morphologically, the ERM bacterium is a 1.0×2.0 to 3.0 μm, gram negative, monotrichously flagellated rod which forms a smooth, circular, raised, entire, nonflourescent, nonpigmented colony on nutrient agar with a buterous type of growth. It is nonaerogenic, fermentative, cytochrome oxidase negative, catalase positive, and typical of enteric bacteria in general and is indole negative, methyl red positive, Voges-Proskauer negative, and citrate positive. This brings it rather closely down to the Erwinia group of the family Enterobacteriaceae. Serologically, antigenic similarities have been found with the somatic O antigens of atypical Erwinia groups 26 and 29 (Ross et al., 1966; Cisar, 1972).

MORTALITY AND TRANSMISSION

Enteric red mouth disease has been known to cause mortality in all trout and salmon. The most susceptible host seems to be rainbow trout, with mortality commonly running from 25 to 75 percent during the course of an untreated epizootic. Populations of brook, *Salvelinus fontinalis,* and brown trout, *Salmo trutta,* seem to be more resistant, with 5 to 10 percent mortality common. The bacterium has not been isolated from warm-water fish, even under experimental laboratory induction. The disease is commonly endemic and survives for long periods of time in organically rich waters and, consequently, lends itself quite well to enzootic infection under intensive culture situations. It is also commonly found as a clinically asymptomatic carrier-state infection.

Transmission of ERM occurs through the water from feces of infected fish. Pathogenesis of infection varies from peracute to chronic depending upon the temperature, stress, species, age, and so forth. Peracute to acute infection usually occur in the spring and early summer, usually in young-of-the-year fish, during periods of rising water temperatures, and increased handling stress. Mortalities usually commence 4 to 8 days following exposure and run between 50 and 70 percent during a 30- to 60-day course of clinical infection. Acute to subacute infections usually occur in yearling fish in the fall and early winter with declining water temperatures. Mortality usually runs about 10 to 50 percent in a 2- to 6-month period. Chronic infection results in very low levels of mortality, approximately 10 percent. However, these losses are often incurred in very valuable market-sized or mature brood stock fishes and, economically, a 10 percent mortality can be significant.

CLINICAL SIGNS AND PATHOLOGY

The clinical pathology of ERM is quite characteristic of bacterial hemorrhagic septicemias in general. By definition, ERM could best be referred to as a bacterial hemorrhagic septicemia of salmonid fishes, caused by the ERM bacterium, having a very well defined geographical range of distribution and endemic occurrence, and capable of inflicting severe mortality.

The common external clinical manifestations include subcutaneous hemorrhaging along the base of the fins and in or about the oral cavity and anus. Exophthalmos is brought about by tissue edema. This condition is noted to often start unilaterally and develop to a bilateral involvement (Fig. 1). Histopathological examination demonstrates an edematous type of lesion of the choroid gland of the eye with an intraocular accumulation of fluid. This edema and increased intraocular fluid pressure induces the exophthalmic condition and eventually results in rupture of the eye with ensuing lens opacity and blindness (Fig. 2). Infected fish will physiologically darken in color and are easily identified in populations suspected as infected, particularly as carriers.

Internal gross pathology is distinguished by petechial hemorrhage of the visceral organs and tissues including the liver, pancreas, adipose tissues, swim bladder, various coelomic mesenteries, and body musculature. Gross tissue edema is noted in the kidney, liver, and spleen along with hemorrhagic reddening of gonadal tissues and the distal ends of the pyloric caeca (Figs. 3-6). The gross pathology of the lower intestine is probably the most significant clinical diagnostic sign of ERM. The intestine becomes inflamed, flaccid, translucent, hemorrhaged, and distended with a serosanguineous yellow mucoid material consisting of necrotized intestinal mucosa heavily loaded with the pathogen.

The histopathology is typical of a hemorrhagic septicemic type of infection and, in the acute form, the bacterium is commonly found in the peripheral blood (Fig. 7). The hematological picture is characterized as an acute macrocytic, hypochromic anemia with leukopenia, resulting from necrotic destruction of hematopoietic and reticuloendothelial tissues, and an increased clotting time as well as a plasma aproteinemia attributed to glomerular nephritis and necrosis of the intestinal mucosa. This condition results in a decrease of the plasma colloid osmotic pressure producing gross systemic tissue edema as well as disruption of the ionic balance.

The acute form of the disease is distinguished by loss of capillary structural integrity, tissue edema, and loss of osmotic and ionic homeostasis. This condition results in a rapid course of infection of 4-10 days with minimum gross clinical pathology. The chronic form is characterized by localized tissue hemorrhage and necrosis, a decline in nutritional condition, and secondary infection. Petechiation results from a loss of capillary integrity and erythrocytic congestion of capillary beds and blood sinusoids (Fig. 8). The intestinal tract demonstrates a progressive necrosis and sloughing of the mucosa proceeding down to the stratum compactum (Fig. 9). This situation is highly conducive to secondary infection and complication of the clinical picture by multiple etiologies.

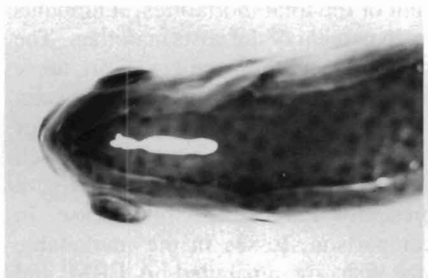


Figure 1.—Bilateral exophthalmos in a rainbow trout, *Salmo gairdneri*, due to gross tissue edema caused by acute septicemic infection with enteric red mouth disease.

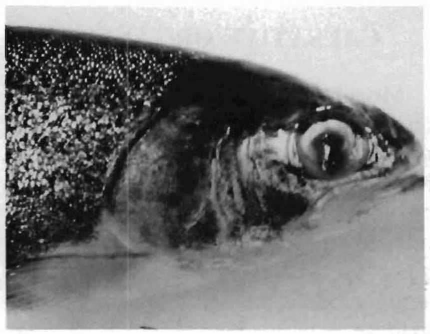


Figure 2.—Rupture of the eye, lens opacity, and blindness in a rainbow trout, *Salmo gairdneri*, due to increased intraocular fluid pressure and edema caused by acute septicemic infection with enteric red mouth disease.

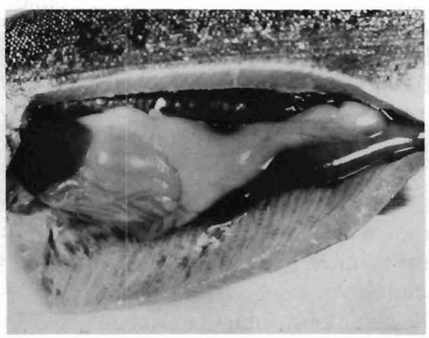


Figure 3.—Generalized petechiation of visceral organs and tissues of a rainbow trout, *Salmo gairdneri*, due to acute septicemic infection with enteric red mouth disease.

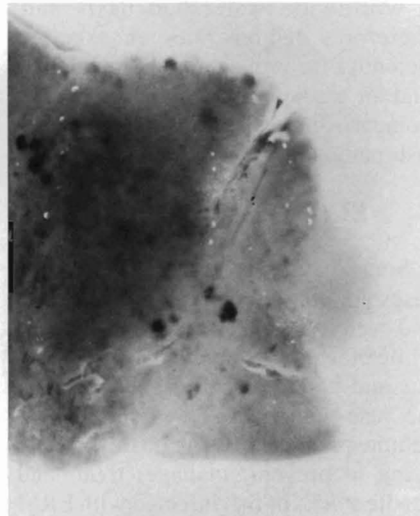


Figure 4.—Petechial hemorrhage in the liver of a rainbow trout, *Salmo gairdneri*, due to acute septicemic infection with enteric red mouth disease.

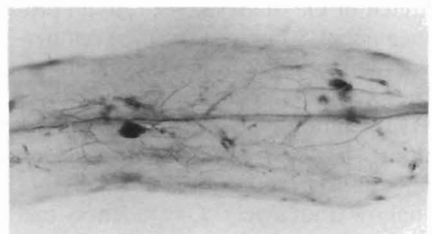


Figure 5.—Hemorrhagic plaques in the swim bladder of a rainbow trout, *Salmo gairdneri*, due to an acute septicemic infection with enteric red mouth disease.

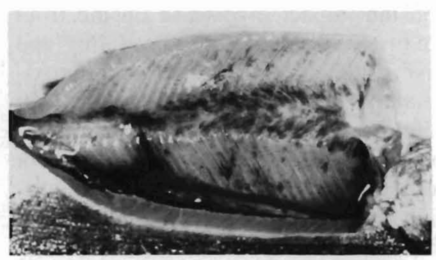


Figure 6.—Petechial hemorrhage of the lateral musculature and visceral mesentery of a rainbow trout, *Salmo gairdneri*, due to an acute septicemic infection with enteric red mouth disease.

remaining there for more than 102 days. The relative intestinal recovery rate and presence of the pathogen within the infected populations was also shown in Figures 12 and 13 to be of a cyclical nature, with a periodicity of 36 to 40 days. Only 25 to 50 percent of the asymptomatic carrier infections established would be clinically identified for inspection and certification purposes by using classical attempts at isolation from the kidney and spleen during the low points of the cycle.

Cyclical Nature of Carrier Infections

On the basis of pathogen recovery, gross pathological changes, and mortality rates, it appears that a regular 36- to 40-day cycle of intestinal shedding of the ERM bacterium occurs and precedes the recurrence of systemic involvment and mortality by 3 to 5 days. This type of cyclical shedding could precipitate continuous recurrent mortality in a naturally infected susceptible population throughout the year. The actual periodicity and mortality levels of these shedding cycles would be altered by seasonal variations in water temperatures, loading factors, handling, and other stresses as well as natural resistance and immunity of the population. This hypothesis is substantiated by the observations of McDaniel (1971), who demonstrated cyclical mortality patterns of similar periodicity to occur throughout the year in a large, untreated hatchery population chronically infected with ERM disease (Fig. 14).

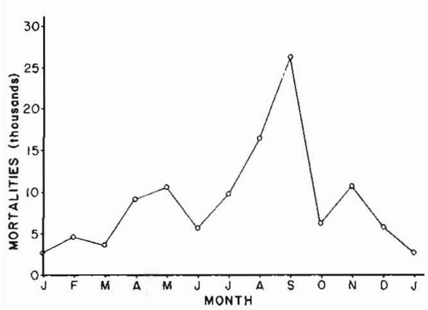


Figure 14.—Monthly mortality rates attributed to endemic enteric red mouth disease infection of an untreated hatchery population of rainbow trout, *Salmo gairdneri*, (from McDaniel, 1971).

Many demonstrated aspects of the clinical course of induced ERM infection have the classical appearance of an acquired protective immune response. In particular, the decrease in mortality and relative rates of gross pathological changes ocurred 14 to 21 days postinfection. This time period corresponds to the expected lag phase and logarithmic induction period of a protective immune response at 14.5°C. Electrophoregram analysis demonstrated a quantitative serum macroglobulin response as shown in Figures 10 and 11. However, this quantitative change in serum macroglobulin could not be detected as agglutinating or precipitating humoral antibodies, indicating the possible involvement of a protective monovalent or incomplete immune globulin fraction, possibly functioning in an opsonizing or cytophilic capacity. An induced cellular immunity is most probably responsible for the rapid clearance of the pathogen from the reticuloendothelial tissues and its localization in the lumen of the lower intestine where it is comparatively removed from macrophage and immunoglobulin activity.

This insight into the establishment of a clinically asymptomatic carrier infection and its demonstrated cyclical nature of shedding could find direct application to management practices of the disease in terms of the timing of handling stress, loading factors, and other "controllable stress." The use of antibiotic or chemotherapeutic agents with a high retention time in the lumen of the gut is indicated rather than other more rapidly absorbed agents for treatment of the carrier condition. Detecting the condition by classical isolation and identification methods (particularly if the lower intestine is not sampled) is difficult, and this may seriously affect surveillance and certification operations. The cyclical nature of the carrier infection also indicates the need for a paired sampling at a 15-day interval to achieve maximum diagnostic efficiency.

Identifying Carriers

Of major consequence to current programs of inspection, certification, and control is the ability to efficiently diagnose clinical carrier infections. Present methods of classical isolation and identification of the pathogen are wholly inadequate in terms of sensitivity, logistics, time frame, and economics. Programs of this type often involve large numbers of fish which must be certified free of particular disease agents but are often highly valuable and cannot be sacrificed as in the case of brood stocks.

Development of the Passive Agglutination Test

As a result of my experience with the U.S. Public Health Service Center for Disease Control's sylvatic plague surveillance program, I have developed a highly sensitive and specific means of serological disease surveillance requiring a minimum of lethal sampling. The procedure is based upon a passive or indirect agglutination test using microtiter techniques.

The antigenicity of the ERM organism was examined in detail. A pH 6.4 boiled aqueous extraction of a washed 18-hour ERM culture was found to be the most antigenically complete soluble fraction of the organism. Many different substrate particle systems, including latex, bentonite, charcoal, fresh erythrocytes, and fixed erythrocytes were incorporated into the test. Maximum sensitivity was obtained with fresh citrated sheep erythrocytes which were tanned and sensitized with 10 mg% antigen protein at pH 6.4. Test sera were diluted in normal physiological saline with normal rabbit serum added at 1:100. Other substrate particle systems were found to be somewhat less sensitive but could be lyophilized and stored for long periods of time.

Figure 15 demonstrates the agglutination patterns achieved. Note the very sharp end points and negative buttons. Titers are easy to pick out, and the test is well adapted to either screening or titering test sera. In addition to the passive agglutination test, a paired inhibition dilution series utilizing 20 mg% antigen protein added to the serum diluent was run. The inclusion of the

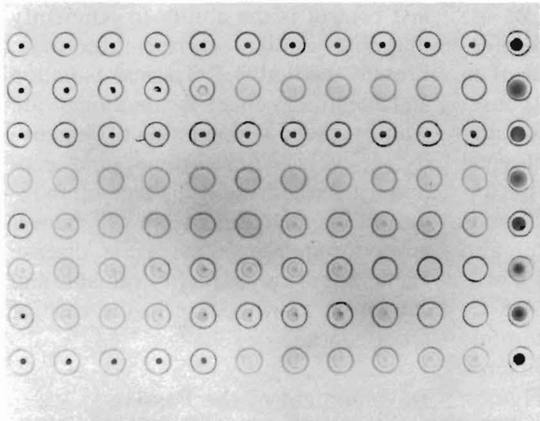


Figure 15.—Typical results of the micromodified passive agglutination test demonstrating diffuse patterns of positive agglutination and negative buttons as well as end point titers of sera dilution series.

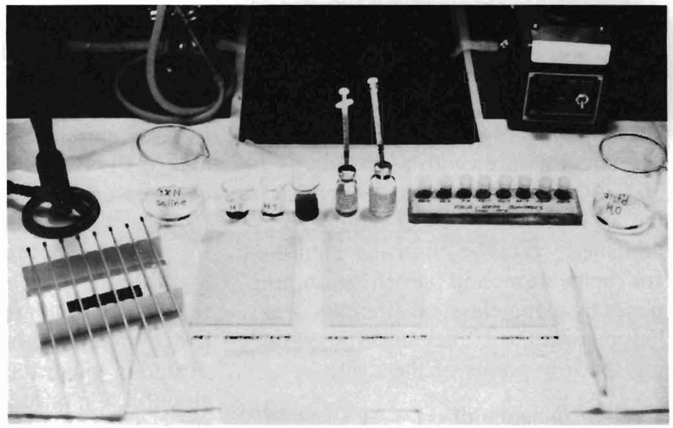


Figure 16.—Basic materials necessary for the performance of the micromodified passive agglutination test applicable for screening of large numbers of individual small volume sera for the presumptive serological evidence of specific disease association.

paired inhibition test gave complete control over any nonspecific agglutinating activity for each sample tested.

This type of test lends itself to the rapid field screening of large numbers of individual small volume sera for presumptive evidence of specific disease association at a minimum cost and without sacrifice of fish over 15 cm. Utilizing an unsophisticated hand-operated system costing $100, a reasonably good technician can screen about 500 fish in an 8-hour day against a variety of diseases (Fig. 16). The test can also be readily automated for even greater efficiency. Results can be read immediately following centrifugation or after 2-4 hours of incubation.

Field Application of Passive Agglutination Test

In the fall of 1972, the passive agglutination procedure was field tested in the Hagerman Valley. The fish stocks of eight stations having a history of ERM, but no recent epizootics, and a single station undergoing an active epizootic were examined. Station stocks were first sampled in September, just at the end of the usual seasonal peak in mortality attributed to ERM disease, and then reexamined 60 days later in November.

Initial serological screening resulted in a 38.2 percent incidence of positive titers for the infected station stocks and a 7.7 percent incidence for all other stations combined. Positive serum titers during this period ranged from 1:16 to 1:32,768. The actual individual titer distributions are summarized in Figure 17 for the two populations. A definite natural split in high and low level titers occurred. High level natural titers of 1:512 or greater (shaded areas of Fig. 17) constituted 19.4 percent of all positive titer (shaded and unshaded combined) from the clinically infected station and 30.8 percent of all positive titers from all other nonclinically infected stations combined during the first sampling date.

As summarized in Figure 18, an 8.4 percent incidence of positive titers for the infected station and an 8.8 percent incidence of positive titers for the other stations were found at the second sampling date. When the natural break between the high and low level titers was examined for the second set of data, high titered sera were absent from the infected station and constituted 21.3 percent of the positive sera from the other stations combined.

It was postulated that the natural break between high titered and low titered sera is indicative of the difference between subacute to chronic clinical infections and the presence of an established carrier population, respectively.

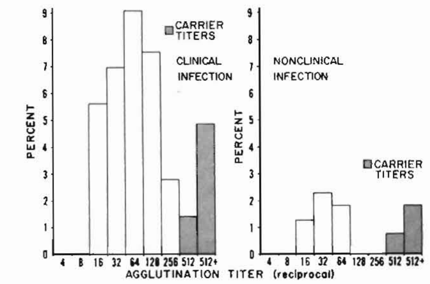


Figure 17.—Natural distribution of serum passive agglutinin titers from hatchery populations of rainbow trout, *Salmo gairdneri*, surviving a recent clinical epizootic of enteric red mouth (ERM) disease (left) and those not clinically infected but grown in an endemic area of ERM infection (right), as sampled in September 1972.

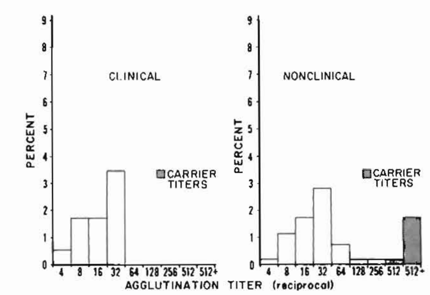


Figure 18.—Natural distribution of serum passive agglutination titers from hatchery populations of rainbow trout, *Salmo gairdneri*, surviving a recent clinical epizootic of enteric red mouth (ERM) disease (left) and those not clinically infected but grown in an endemic area of ERM infection (right), as sampled in October 1972.

This hypothesis is based upon the premise that continued presence of the pathogen over a long period of time, as occurs in an established carrier infection, is necessary to induce high levels of serum agglutinins. The lower titered grouping would then include those survivors which still may harbor the pathogen but have not yet established classical carrier states of infection. Such a hypothesis is supported by the fact that the relative rates of occurrence of the high titered grouping remained constant in the combined stations sampled 60 days apart as shown in the shaded areas of Figures 17 and 18. The 2.7 percent and 2.0 percent respective proportions of the populations seem to demonstrate a constant level of carrier incidence which is consistent with levels of carrier incidence reported for other related diseases.

The overall results from the infected station, as shown in Figures 17 and 18, indicate an initially high rate of infection with a proportionally large number, 6.3 percent, of high titered presumptive carrier fish being present. The second sampling indicated a 31 percent decrease in positive screen titers and the complete lack of any high titered group of sera. This situation could be indicative of a resistant recovering population in which true carrier states had yet to be established. This data could also be attributed to the fact that the second sampling was essentially a different lot of fish from those originally sampled, even though the station was the same.

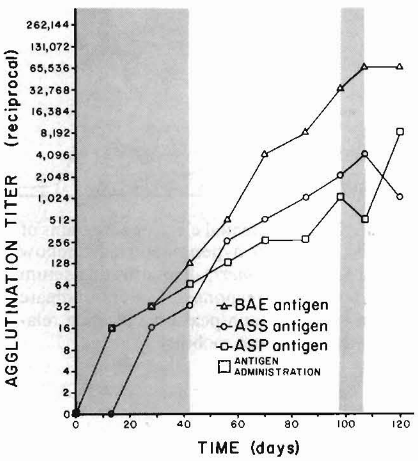


Figure 19.—The serum passive agglutinin response of rainbow trout, *Salmo gairdneri,* to the administration of water soluble antigenic preparations of the enteric red mouth disease bacterium. (BAE, boiled aqueous extract; ASS, ammonium sulfate supernate; ASP, ammonium sulfate precipitate.)

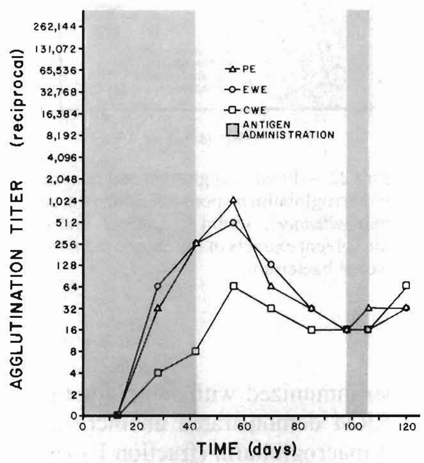
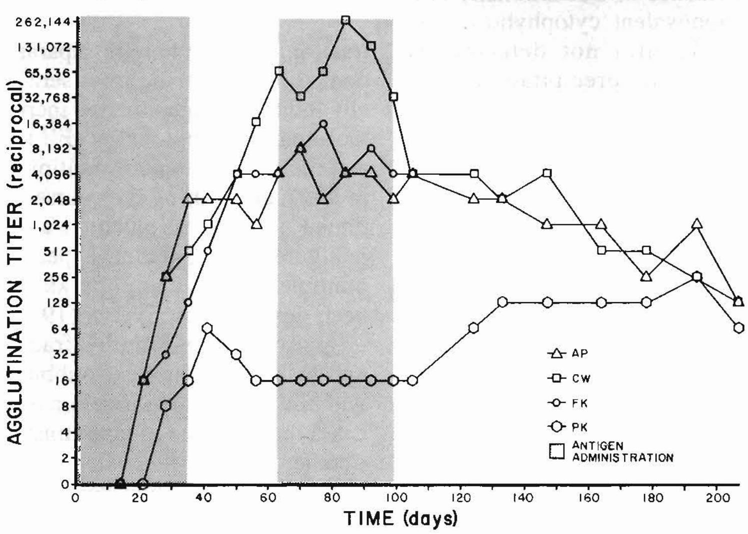


Figure 20.—The serum passive agglutinin response of rainbow trout, *Salmo gairdneri,* to the administration of organic solvent soluble antigenic preparations of the enteric red mouth disease bacterium. (PE, phenol extract; EWE, ether-water extract; CWE, chloroform-water extract.)

Figure 21.—The passive serum agglutinin response of rainbow trout, *Salmo gairdneri,* to the administration of particulate antigen preparations of the enteric red mouth disease bacterium. (AP, alum precipitate; CW, cell wall; FK, Formalin killed; PK, phenol killed.)



INDUCED HUMORAL IMMUNE RESPONSE IN RAINBOW TROUT

Induction of a specific immune response in rainbow trout to immunogenic preparations of the ERM bacterium was examined in terms of its nature and dynamics.

Figures 19 and 20 demonstrate the dynamics of a humoral agglutinin response in rainbow trout to extended parenteral exposure to soluble antigenic preparations of the ERM bacterium. Protein-based water soluble extracts are shown in Figure 19 and carbohydrate-based organic solvent extracts are shown in Figure 20. Various preparations included a boiled aqueous extract (BAE), ammonium sulfate supernate (ASS), ammonium sulfate precipitate (ASP), phenol extract (PE), ether-water extract (EWE), and chloroform-water extract (CWE). Humoral passive agglutinating antibodies against protein-based soluble antigens were first detected 13 days after intiation of the first of two series of weekly injections. Specific passive agglutination titers to organic solvent extracts were initially detected in 28 days. All serum agglutinin titers were shown to rise throughout the initial period of antigenic stimulation, but when injections were discontinued, titers to lipopolysaccharide antigens declined while titers to protein antigens continued to rise. Freund's complete adjuvant was used in the first two injections of all preparations.

A second series of weekly injections, commencing on experimental day 98, was shown to have an inductive effect on specific titers of all antigens but not to the extent of a true anamnestic response. Maximum serum passive agglutinin titers of 1:65,531 were obtained from a protein-based antigen (BAE) at 106 days. Comparatively poor responses were obtained from the lipopolysaccharide preparations. The EWE and CWE antigens were extremely toxic to rabbits due to their lipopolysaccharide endotoxin components but were found to have no toxic effect upon trout at the levels administered.

Rainbow trout were also injected with various particulate antigen preparations of ERM including an alum precipitate (AP), washed cell wall suspension (CW), and [1]Formalin-killed (FK) and phenol-killed (PK) whole cells. Humoral passive agglutinins to these particulate antigens were detected at 21 to 28 days into the first series of weekly injections as shown in Figure 21. The CW preparation induced the highest passive agglutinin response with a maximum titer of 1:262,144 in 84 days. The FK and AP antigens gave much the same response. The PK preparation induced a poor serum passive agglutinin response while a heat-killed preparation failed to elicit any type of a passive agglutinin response at all. The high passive agglutination titers induced by various particulate antigens seemed to reach a common maximum titer of 1:4,096 following discontinuation of injections. From this point, the various titers declined similarly at an average rate of 9.8 percent per month and were detectable for more than 9 months.

Quantitative analysis of serial electrophoregrams from rainbow trout

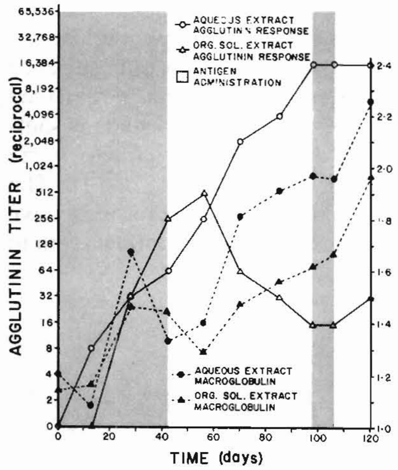


Figure 22.—Passive agglutinin and quantitative macroglobulin response of rainbow trout, *Salmo gairdneri*, serum to aqueous and organic solvent extracts of the enteric red mouth diesease bacterium.

hyperimmunized with soluble antigens of ERM demonstrated an increase in total macroglobulin (fraction F) serum protein during continued antigenic stimulation but no direct correlation could be made to the presence of serum passive agglutinins (Fig.22). However, the macroglobulin response is seen to be directly proportional and responsive to antigenic stimulation and resembles a classical humoral immune response including what appears to be a true anamnestic response at 106 days. This macroglobulin response could be further evidence of a functionally protective monovalent cytophylic or opsinizing globulin not detected by agglutination or precipitation techniques.

Electrophoretic analysis of the time sequence sampling of pooled sera from hyperimmunized rainbow trout produced a normal seven protein component pattern, as shown in Figure 23, consisting of: A prealbumin and albumin complex grouped as peak "A"; a second component, labeled "B," analogous to an alpha-1 peak of lipoprotein; three pseudoglobulin peaks, analogous to alpha-2, beta-1, and beta-2 fractions and called "C," "D," and "E"; and a euglobulin or gamma complex of peaks labeled as the "F"

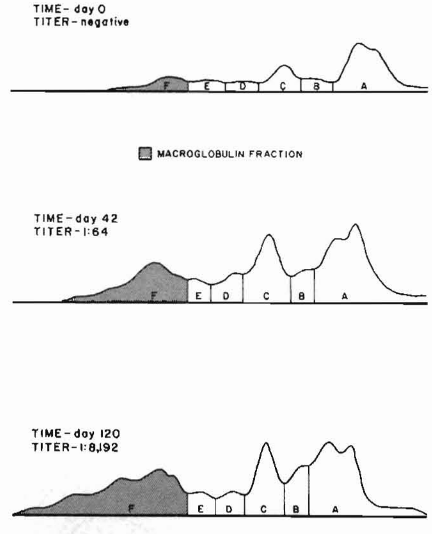


Figure 23.—Sequential electrophoregrams of pooled sera from hyperimmunized rainbow trout, *Salmo gairdneri*, demonstrating serum macroglobulin response. Letters indicate major serum protein peaks based upon relative electrophoretic mobility.

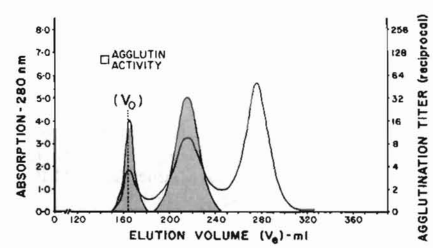


Figure 24.—Gel filtration chromatographic fractionation and passive agglutinin activity determination of hyperimmune mammalian (rabbit) serum proteins.

fraction. Electrophoretic separation of pooled hyperimmune trout sera generally indicated a quantitative increase of the anodic macroglobulin "F" fraction and the development of multiple peaks in this region during the course of continued antigenic stimulation as shown in Figure 23. The gradual appearance of multiple macroglobulin peaks has also been described by Evelyn (1971).

Analysis of gel filtration fractions of anti-ERM hyperimmune rabbit serum with the passive agglutination test indicated the presence of agglutinating antibodies in both the 18S-19S and 7S fractions of the rabbit serum as indicated in Figure 24. However, specific

[1]Reference to trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

agglutinin activity was found in only the macroglobulin fraction of the hyperimmune trout serum as shown in Figure 25. No shifts in specific immune activity among effective molecular weight globulin fractions were found to occur in the salmonid sera during extended periods of immunization. Some consistent but seemingly nonspecific activity was also found in the prealbumin fraction of the trout serum.

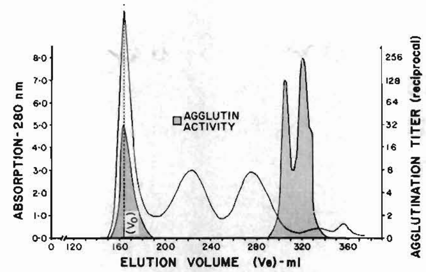


Figure 25.—Gel filtration chromatographic fractionation and passive agglutinin activity determination of hyperimmune rainbow trout, *Salmo gairdneri*, serum proteins.

SUMMARY

The ERM bacterium has been shown to be a highly infectious and economically significant pathogen of salmonid fishes, particularly under the stresses of intensive culture. Due to its present limited but expanding geographical distribution, it is of primary importance that preventative measures be taken to limit the further dissemination of the disease. Such preventative measures and treatment should take into account the presented findings concerning the epidemiology and pathogenesis of the disease and, in particular, the asymptomatic carrier state of infection. Programs of inspection, certification, and surveillance, based upon presumptive serological screening procedures adapted to large numbers of individual small volume sera with minimum lethal sampling, give promising results for disease diagnosis, surveillance, and detection of clinically inapparent carrier infections.

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