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VIBRIOSIS IN RAINBOW TROUT

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Abstract: An epizootic of sub-acute vibriosis occurred among a population of rainbow trout *(Salmo gairdneri)* during experimental acclimatization to seawater. The causative organism was identified as *Vibrio anguillarum* and its characteristics are listed.

INTRODUCTION

There are numerous reports of vibrio infections in marine and migratory fish and the host range and geographic distribution have been listed by Anderson and Conroy.[1] Vibriosis, possibly due to marine vibrios, has also been reported from captive fish in a freshwater environment.[17,18]

The present communication describes an outbreak of vibriosis among rainbow trout *(Salmo gairdneri)* during experimental acclimatization from fresh to seawater.

The epizootic

The epizootic occurred among a group of 200 rainbow trout, taken from a larger batch of 2,000 fish, which had been received from a commercial trout farm and quarantined in freshwater for 14 days. All fish were of a similar size and weight: mean fork length — 17.5 cm; mean weight — 65g.

Acclimatization involved placing fish in 20% seawater for 4 days, then increasing the proportion of seawater by a further 10% after each period of 4 days acclimatization until 100% seawater was reached. Fish were divided between two 400 litre fibreglass tanks, each with a water flow of 2 litres per minute and both being well aerated. The seawater used in the experiments had been obtained at high tide for greater clarity and stored for 2 days prior to use. Water temperature varied during the period of acclimatization within the range 10-13 C. Fish were fed a mixed diet of commercial pelleted trout food and *Calliphora* larvae.

Skin lesions were first noticed on several fish shortly before the initial mortality occurred on day 15 (seawater concentration 60%). Despite a gradual increase in mortality, acclimatization was continued until by day 27 (100% seawater), 41 fish had died. All remaining fish exhibited external signs of disease and all were dead by day 40. The residual population of 1,800 fish, still held in freshwater, remained healthy.

All affected fish showed similar clinical signs. Externally, lesions were present on both body flanks, varying in severity from small areas of scale loss to large ulcers. Large ulcers were characterized by central areas of haemorrhagic muscle and by extensive peripheral areas of scale loss. Internally, numerous petechial haemorrhages were present on the walls of the coelomic cavity and intestinal tract. The spleen was congested and extremely friable, while the kidney and liver appeared normal.

MATERIALS AND METHODS

Isolation procedure

A bacteriological examination was made of four diseased fish and two taken from the healthy population. Tissue was removed aseptically from the lesion (diseased fish only), and from heart blood, spleen, liver, kidney and intestinal tract. It was streaked on two types of tryptone

soya agar (TSA) (Oxoid Ltd.): one prepared with distilled water, the other with 75% aged seawater in distilled water. All plates were incubated at 22C for 4 days. Smears from these tissues were also made and stained by Gram's method.

The main purpose of this paper was to identify the causative agent of the epizootic and not to ascertain its origin. However, an attempt was also made to discover the source of the offending organism by culturing the seawater used for acclimatization as well as the pelleted fish food.

Examination of seawater

Samples were taken from the seawater storage tank and diluted with physiological saline in a range 1:10 to 1:10,000. Duplicate 1 ml samples of each dilution were spread on both types of TSA plates and incubated at 22C. Colonies were counted at 48 hours and those resembling vibrios were subcultured and identified.

Examination of food

In addition to the food used during the present procedure, two other commercial types of pelleted trout food were included in the study. One gram samples of food were thoroughly crushed with a sterile pestle and mortar and then added to 500 ml of tryptone soya broth (Oxoid Ltd.) made with 75% aged seawater. Broths were incubated at 22C for 48 hours and subcultured on to both types of TSA plates as before.

Characterization tests

A more detailed report on the bacteriological and serological characteristics of the present isolate will be published shortly: therefore, only a brief summary of the methods used for characterization are given here.

Flagella were stained by the method of McCarthy and Stevenson.[14] Butanediol production was tested for as described by Bullock.[2] Deoxyribonuclease activity was determined on deoxyribonuclease test agar (Oxoid Ltd.), by flooding the growth with 0.1 N hydrochloric acid. Sensitivity to antibiotics was assayed on diagnostic sensitivity test agar (Oxoid Ltd.). Tolerance to sodium chloride was estimated by subculture on to TSA plates containing final concentrations of sodium chloride over the range 1-10%.

The following tests were performed by the methods stated: dihydroxy-acetone (DHA) production;[3] lecithinase activity;[6] esterase activity;[4] cadmium sensitivity.[9]

All other characterization tests were performed by standard methods.[5,11]

Histopathology

The following tissues were fixed in 10% buffered formal saline and stained with haematoxylin and eosin: skin lesion, spleen, liver and kidney.

RESULTS

Bacteriology

Gram-stained smears from all diseased fish revealed large numbers of gram-negative rods which exhibited pronounced somatic curvature. These cells were absent from healthy fish.

All culture plates, inoculated with material from affected fish, grew a pure or predominant growth of a gram-negative, curved rod 1.5-2.5μ x 0.4-0.6μ, absent from the healthy fish. The bacterium grew better on TSA prepared with seawater than with distilled water. Tissues from healthy fish were sterile.

The organism was actively motile and possessed polar flagella. An observation, concerning inhibition of motility of *V. anguillarum* strains when suspended in distilled water,[16] was confirmed with the present isolate.

Bacteriological results are shown in Table 1. Since most test substrates contained only 0.5% sodium chloride, and in view of the observed halophilic properties of the organism, all tests with negative results were repeated on sub-

TABLE 1. Characteristics of the vibrio isolate.

Test	Results	Test	Results
Acid from glucose	+	Phenylalanine deamination	—
glycerol	+	Arginine hydrolysis	+
adonitol	—	Ornithine decarboxylation	—
arabinose	+	Lysine decarboxylation	—
cellobiose	+	Haemolysin production	+
dextrin	+	Pigment production	—
dulcitol	—	Urease activity	—
fructose	+	Esterase activity	+
galactose	+	Lecithinase activity	+
inositol	—	Lipase activity	+
inulin	—	Phosphatase activity	+
lactose	—	Catalase activity	+
maltose	+	Oxidase activity	+
mannitol	+	Deoxyribonuclease activity	+
mannose	+	Nitrate reduction	+
raffinose	—	Nitrite reduction	+
rhamnose	—	Citrate utilization	—
salicin	—	Formate utilization	+
sorbitol	+	KCN *	R
sorbose	—	Cadmium sulphate	R
sucrose	+	Vibriostat	S
trehalose	—	Ampicillin 25 *	S
xylose	—	Chloramphenicol 50	S
Sodium chloride sensitivity	6%	Colistin 50	S
Aesculin hydrolysis	+	Fusidic acid 10	R
Starch hydrolysis	+	Kanamycin 5	R
Gas from glucose	—	Methicillin 10	R
Gas from glycerol	—	Nalidixic acid 30	S
Oxidation-Fermentation	F	Neomycin 10	S
Methyl-red test	+	Nitrofurantoin 200	S
Voges-Proskaur	+	Novobiocin 5	S
Butanediol production	+	Streptomycin 25	S
ONPG test	+	Tetracycline 50	S
DHA production	+		
Gluconate oxidation	—		
Caseinase activity	+	* R = Resistant	
Gelatinase activity	+	S = Sensitive	
H_2S production	+	* Antibiotic concentrations are in μgms/ml	
Indole production	+		

strates containing 2% sodium chloride. Using this technique, negative results obtained for the indole test and potassium cyanide sensitivity were reversed.

Although numerous vibrios were isolated from the seawater (total viable count 5.4 x 10^4), the organism involved in the present epizootic was not recovered.

Vibrios were not isolated from either of the three commercial pelleted food diets examined.

Histopathology

Approximately 6 hours had elapsed *post mortem* before tissues could be fixed, consequently in most tissues some autolytic degeneration had occurred. This was particularly noticeable in sections from kidney and liver. However, histopathological signs were sufficiently distinct to provide supporting evidence for a firm diagnosis of sub-acute vibriosis.

Sections through lesions revealed marked muscle necrosis accompanied by numerous inter-fibrillar haemorrhages; congestion of inter-fibrillar vessels and a lack of leukocytic response were also noted. Large numbers of distinctly curved bacilli were present in all tissues from affected fish. Tissues from healthy fish appeared normal.

DISCUSSION

Clinical signs, observed in the present epizootic, were similar to those described elsewhere for vibriosis,[1] except that the boil-like lesions described by Hoshina[12] and Ross *et al*,[16] also in rainbow trout, were not seen. The apparent difference in external signs might be explained on the basis that these two[12,16] epizootics occurred in freshwater, though it is to be noted that Smith[18] observed considerable variation in clinical signs of vibriosis in the same fish species. Whatever the mechanism involved, the reported occurrence of boil-like lesions on rainbow trout, held in freshwater and suffering from vibriosis, indicates the necessity for making a careful examination in order to distinguish vibriosis from furunculosis. Moreover, the external clinical signs noted on some fish in the present outbreak (small areas of descalation) were strikingly similar to those observed on rainbow trout during an outbreak of sub-acute furunculosis in freshwater (McCarthy, unpublished observation). Although it is probably not surprising that physiologically similar bacteria elicit a similar pathological effect in fish, these findings, together with the recent isolation of *A. salmonicida* from a strictly marine host,[8] the sable fish *(Anoplopoma fimbria),* emphasizes the clear necessity for a thorough bacteriological examination in order to distinguish unequivocally between vibriosis and furunculosis.

All captive fish held in seawater are at risk from vibriosis because the organism is present in their environment. Freshwater fish, on the other hand, may be infected by means of contaminated food. Rucker *et al*[17] described a case of vibriosis in rainbow trout, held in freshwater, which had been fed salmon viscera and marine scrap-fish. Ross *et al*[16] also reported a similar outbreak among rainbow trout in freshwater, but here they were of the opinion that because a pelleted food was used it was unlikely to be responsible for the introduction of the pathogen. Clearly, however, the organism behaved like a marine bacterium.

Although it was not possible to establish it, seawater was most likely the source of the organism. Pelleted diets, although containing marine fish products, were a less likely source because of the probable destruction of most bacteria due to heating during processing.

Wherever the organism came from, the present epizootic clearly illustrates that freshwater fish are particularly susceptible to vibriosis during acclimatization to saltwater, and this indicates the necessity for a close observation of such fish.

The bacterium thought to be responsible for the present outbreak was identified as *V. anguillarum*. The classifica-

tion of *V. anguillarum* has recently been reviewed in some detail by Evelyn[7] and Levin *et al.*[13] The present authors are in agreement with the view of Evelyn[7] that, because of reported variations in the biochemical characters used to distinguish between Nybelin and Smith's[16,18] types A, B and C, this classification can be of little value and should be discontinued. The suggestion of Hendrie *et al*[10] that *V. piscium, V. ichthyodermis* and *V. anguillarum* should be combined as a single species, *V. anguillarum,* is welcomed as this should simplify the identification of vibrios potentially pathogenic to fish.

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