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HEALTH STATUS OF SALMONIDS IN RIVER SYSTEMS IN NATAL. III. ISOLATION AND IDENTIFICATION OF BACTERIA

R. R. BRAGG[1], Department of Poultry Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110

ABSTRACT

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Both pathogenic and non-pathogenic bacteria were isolated from fish, both salmonid and non-salmonid, from selected river systems in Natal. *Pasteurella pisicida* was isolated for the first time from fish in South Africa. The isolation of *Yersinia ruckeri, Aeromonas salmonicida,* and *Edwardsiella tarda* were recorded for the first time from fish in Natal. *A. hydrophila* and *Flexibacter columnaris* were found to be widespread throughout the river systems in Natal. The *Streptococcus* species which caused serious disease problems in trout in the Cape Province and Transvaal was not isolated from any of the fish examined in Natal.

INTRODUCTION

Little is known of the bacteriological status of salmonids in southern African river systems. There is, however, some literature on the isolation of various species of bacteria from fish kept under hatchery conditions in this country.

The most serious bacterial pathogen which has been isolated from trout in this country is a *Streptococcus* species. This bacterium was first recorded in this country in 1979 (Boomker, Imes, Cameron, Naudé & Schoonbee, 1979) and the bacterium was re-isolated again in 1985 and 1986 (Bragg & Broere, 1986) when it caused very severe mortalities in a number of trout hatcheries in the Transvaal and one hatchery in the Cape. There have never been any reported cases of streptococcosis in Natal and the isolation of this bacterium has never been reported from fish from that province.

Yersinia ruckeri, the causative agent of enteric redmouth, has been isolated from a population of diseased fish in the Transvaal (Bragg & Henton, 1986). This bacterium has only been isolated from fish in the Transvaal and has never been isolated from fish in Natal or the Cape Province.

Aeromonas salmonicida, the causative agent of furunculosis, has been isolated from trout reared in sea water in the Cape Province (Boomker, Henton, Naudé & Huchzermeyer, 1984) and from rainbow trout in the Transvaal (Bragg & Combrink, 1988). There has never been any reports of furunculosis from fish in Natal. *A. hydrophila* has been found to be widespread throughout this country (Bragg, Combrink & Broere, 1986) and has been isolated from fish, both salmonid and non-salmonid from the Transvaal, Cape and Natal.

Flexibacter columnaris has been reported previously in South Africa (Du Plessis, 1952; Lombard, 1968) and has also been isolated from Natal (Bragg, Todd & Lordan, 1988, unpublished data). There is also a number of unconfirmed reports of columnaris in the Transvaal and Natal and it would appear that the bacterium is widespread throughout the country.

These bacteria have all been isolated from diseased fish. There has never been a bacteriological survey of healthy populations of fish in either the rivers or production sites in this country. This third part of a three part series, covers the bacteriological aspects of an in depth microbiological survey of salmonids and non-salmonids from various river systems in Natal.

[1] Previously of the Fish Disease Unit, Veterinary Research Institute, Onderstepoort 0110

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MATERIALS AND METHODS

Collection of fish from rivers, production sites and dams

The methods used for the collection of fish as well as the sites from where fish were collected were the same as those described in Part 1 (Bragg 1991a).

Processing of fish for the collection of bacteriological samples

Fish collected from sites 1 to 17 (Table 1 of Part 1, Bragg, 1991a) were examined for the presence of the trout pathogenic strain of *Streptococcus* by isolation and identification of the bacterium (Bragg, Todd, Lordan & Combrink, 1989) and for *Y. ruckeri* by the indirect immunofluorescent antibody technique (IFAT). Fish collected from samples 18 to 38 (Table 1 of Part 1, Bragg, 1991a) were subjected to a more extensive bacteriological examination. After samples had been collected for parasitological examination (Bragg, 1991a), a swab of the body surface of the fish was inoculated in Shieh broth (Shieh, 1980) which facilitates the isolation of *F. columnaris* (Song, Freyer & Rohovec, 1988).

The fish were subsequently cut open to reveal the viscera. Swabs of the gut were collected and plated onto blood tryptose agar (BTA), Waltman-Shott (WS) agar, a selective medium for *Y. ruckeri* (Waltman & Shotts, 1984) and Rimler-Shott (RS) agar which is selective for *Aeromonas* spp. (Shotts & Rimler, 1973). Swabs were also collected from the gut and used to inoculate the liquid media (Shieh and *Streptococcus* selective broth). Swabs of the gut were also collected and used to make smears on clean glass microscope slides.

The viscera were removed to reveal the kidney and swabs were collected and inoculated onto the same media as those used for the isolation of bacteria from the gut. The only liquid medium to be inoculated from the kidney was the *Streptococcus* selective broth. Smears of the kidney were also made on glass microscope slides.

Isolation of bacteria

Upon return to the laboratory, the solid and liquid media were incubated at room temperature and examined for 5 days for signs of growth. If any growth was observed in the *Streptococcus* selective broth, subcultures were made onto tetrazolium agar plates (Bragg *et al.*, 1989) and incubacted for up to 3 days. All small red colonies on the tetrazolium agar plates were plated out onto BTA plates and identified. The Shieh broth was subcultured onto Cytophage agar (Ordal & Rucker, 1944) and incubated at room temperature. Any yellow colonies found on the Cytophage agar were plated out onto BTA plates and identified.

The number of different colony types on the BTA plates were recorded and a representative of each colony type was plated out onto BTA plates and identified.

Any greenish colony, or a colony with a halo, representing Tween 80 hydrolysis on the WS plates (Waltman & Shotts, 1984) were plated onto BTA plates, incubated and identified. Any colonies on the RS agar were plated out onto BTA plates, incubated and identified.

Fluorescent antibody tests

An indirect fluorescent antibody test (IFAT) to detect the presence of the trout pathogenic *Streptococcus* sp. (Bragg, 1988a) was carried out on the smears which were made from the gut and kidney of the fish. An IFAT to detect the presence of *Yersinia ruckeri* was carried out on the smears made from the kidney and gut of the fish. The technique used was similar to that used to detect the trout pathogenic *Streptococcus* sp., except that *Y. ruckeri* specific antibodies, which were produced in this laboratory, were used to overlay the smears.

A direct immunofluorescent antibody technique (DFAT) was carried out to detect the presence of *Renibacterium salmoninarium* (Evelyn, Ketcheson & Prosperi-Porta, 1981) in smears of the kidney of fish collected from samples 18 to 32 (Table 1 of Part 1, Bragg, 1991a). The FITC labelled antibodies against *R. salmoninarium* were kindly supplied by Prof. Klontz of Moscow University, Idaho, USA.

Identification of bacteria

A Gram stain was done on each of the isolates. Yeast species, gram positive rods and gram negative cocci were not identified further, but were recorded. The gram positive cocci were divided into the *Staphylococcus* group and the *Streptococcus* group according to the catalase and oxidase reactions. The *Staphylococcus* group was not identified any further. A plate agglutination test using rabbit raised antibodies against the trout pathogenic *Streptococcus* sp. (Bragg, 1988a) was carried out on the *Streptococcus* group. *Streptococcus* spp. which were positive on the plate agglutination test were recorded as the trout pathogenic *Streptococcus* sp., while those which were negative were recorded as '*Streptococcus* spp.'

All gram negative rods were divided according to their catalase and oxidase reactions. The bacteria were identified according to the tables presented in Austin & Austin (1987).

RESULTS

Collection of fish from rivers, production sites and dams

The number of fish and the localities of fish caught can be seen in Part 1 of this series (Bragg 1991a).

Fluorescent antibody test

No trout pathogenic *Streptococcus* sp. or *R. salmoninarium* could be detected on any of the smears. When the IFAT was carried out using antibodies against *Y. ruckeri,* positive fluorescence was detected in some of the smears collected from fish in the Mooi, Bushmans, Polela, Umzimkulu and Mahai/Tugela rivers (Table 1).

Isolation and identification of bacteria

A large number of bacteria was isolated from both the gut and the kidney of fish collected from various sites during this survey. A number of these bacteria were found to be pathogenic or potentially pathogenic for trout (Table 2). A number of non-pathogenic bacteria were also isolated from these fish and these bacteria are listed in Table 3.

TABLE 1 Results of direct and indirect immunofluorescent antibody tests carried out on smears collected from fish during this survey [site number corresponds with the site numbers in Table 1 of Part 1 (Bragg 1991a)]

Site	River	1 (gut)	1 (kidney)	2	3
1	Mooi	–	–	–	N/D
2	Mooi	43,4 %	4,8 %	–	N/D
3	Mooi	41,2 %	27,8 %	–	N/D
4		32,6 %	50,0 %	–	N/D
5		–	–	–	N/D
6	Mooi	33,2 %	11,1 %	–	N/D
7		–	–	–	N/D
8		–	–	–	N/D
9	Umzimkulu	–	–	–	N/D
10	Umzimkulu	N/D	N/D	N/D	N/D
11	Ingwagwana	N/D	N/D	N/D	N/D
12	Umzimouthi	–	–	–	N/D
13	Umzimkulu	–	–	–	N/D
14	Polela	–	–	–	N/D
15	Bushmans	6,3 %	–	–	N/D
16	Bushmans	–	–	–	N/D
17	Bushmans	–	–	–	N/D
18	Mooi	–	–	–	–
19	Mooi	–	–	–	–
20	Mooi	–	–	–	–
21	Mooi	–	–	–	–
22	Bushmans	–	–	–	–
23	Bushmans	6,5 %	–	–	–
24	Bushmans	–	–	–	–
25	Umzimkulu	4,2 %	–	–	–
26	Polela	2,4 %	–	–	–
27	Polela	–	–	–	–
28	Ingwagwana	–	–	–	–
29	Ingwagwana	–	–	–	–
30	Umzimouthi	–	–	–	–
31	Umzimouthi	–	–	–	–
32	Ingwagwana	–	–	–	–
33	Mlambonjwa	–	–	–	–
34	Mlambonjwa	–	–	–	–
35	Mlambonjwa	–	–	–	–
36	Mlambonjwa	–	–	–	–
37	Mahai/Tugela	9,6 %	3,2 %	–	–
38	Mahai/Tugela	15,3 %	4,6 %	–	–

N/D = Not done
– = Negative
1 = *Yersinia ruckeri*, 2 = *Streptococcus* sp.
3 = *Renibacterium salmoninarium*

DISCUSSION

Y. ruckeri was isolated from fish in Natal for the first time as a result of this work. The first indication that *Y. ruckeri* may be present was obtained when fish were collected from Sites 1–3 & 6 (Table 1 of Part 1, Bragg, 1991a). A high percentage of brown trout in the Mooi river, from both above and below the hatchery at Kamberg was found to be positive by IFAT. A much lower incidence of 6,3 % (Table 1) of carriers was detected in the brown trout in the Bushmans river. These findings were not confirmed by isolation and identification of *Y. ruckeri* on this occasion as these samples were collected primarily for the isolation of infectious pancreatic necrosis (IPN) virus (Bragg, 1991b).

During later collection trips, *Y. ruckeri* was isolated from fish collected from four of the eight rivers tested. The bacterium was, however, not isolated from the Mooi river, but was isolated from the Bushmans river with an incidence of 6,6 % from the brown trout. The results of the IFAT and culturing from the Bushmans river correspond exceptionally well. The reason for the negative isolation and IFAT results (Table 1) from the Mooi river during the later collection trips, in spite of high incidences of carriers detected previously by using IFAT, could not be

TABLE 2 List of pathogenic bacteria, and incidence levels, isolated from fish collected as part of the survey [site number corresponds with the site numbers in Table 1 of Part 1 (Bragg 1991a)]

Site	River	1	2	3	4	5	6	7	8	9
1	Mooi	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
2	Mooi	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
3	Mooi	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
4		N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
5		N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
6	Mooi	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
7		N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
8		N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
9	Umzimkulu	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
10	Umzimkulu	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
11	Ingwagwana	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
12	Umzimouthi	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
13	Umzimkulu	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
14	Polela	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
15	Bushmans	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
16	Bushmans	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
17	Bushmans	N/D	–	N/D	N/D	N/D	N/D	N/D	N/D	N/D
18	Mooi	–	–	–	56 %	12 %	–	20 %	–	–
19	Mooi	–	–	–	60 %	10 %	–	10 %	–	–
20	Mooi	–	–	–	50 %	–	–	13 %	–	–
21	Mooi	–	–	–	43 %	–	–	–	–	–
22	Bushmans	–	–	20 %	27 %	7 %	–	–	–	–
23	Bushmans	6,6 %	–	35 %	27 %	7 %	–	–	–	–
24	Bushmans	–	–	11 %	43 %	11 %	–	–	–	–
25	Umzimkulu	5 %	–	15 %	53 %	5 %	5 %	10 %	–	33 %
26	Polela	2 %	–	–	29 %	–	17 %	–	–	45 %
27	Polela	–	–	–	100 %	–	–	–	–	–
28	Ingwagwana	–	–	–	8 %	8 %	40 %	–	–	8 %
29	Ingwagwana	–	–	10 %	70 %	–	20 %	–	–	–
30	Umzimouthi	–	–	–	39 %	6 %	11 %	–	–	28 %
31	Umzimouthi	–	–	7 %	21 %	14 %	–	–	–	–
32	Ingwagwana	–	–	19 %	10 %	24 %	10 %	5 %	–	10 %
33	Mlambonjwa	–	–	–	81 %	14 %	5 %	6 %	–	19 %
34	Mlambonjwa	–	–	–	67 %	–	17 %	–	–	–
35	Mlambonjwa	–	–	10 %	70 %	10 %	–	–	–	–
36	Mlambonjwa	–	–	–	42 %	42 %	17 %	8 %	8 %	–
37	Mahai/Tugela	17 %	–	–	42 %	75 %	–	–	–	–
38	Mahai/Tugela	40 %	–	–	20 %	50 %	–	–	–	–

N/D = Not done
– = Negative
1 = *Y. ruckeri*
2 = Trout pathogenic *Streptococcus* sp.
3 = *A. salmonicida*
4 = *A. hydrophila*
5 = *F. columnaris*
6 = *Vibrio* spp.
7 = *Pseudomonas* spp.
8 = *E. tarda*
9 = *P. pisicida*

TABLE 3 List of non-pathogenic bacteria from the eight river systems tested during this survey

Isolate	1	2	3	4	5	6	7	8
Bacillus sp.	+	+	+	+	+	+	+	+
Enterobacter sp.	+	+	–	–	–	–	+	–
Acinetobacter sp.	+	+	+	+	+	+	+	+
Klebsiella sp.	+	+	–	+	+	+	+	+
Chromobacter sp.	+	+	–	–	–	–	–	–
Citrobacter sp.	+	+	–	–	–	–	+	+
Erwinia sp.	+	+	–	–	–	+	+	+
Serratia sp.	+	+	+	+	+	+	+	+
Staphylococcus sp.	+	+	+	+	+	+	+	+
Proteus sp.	+	+	–	–	–	–	–	–
Plesiomonas sp.	–	+	–	–	–	–	+	–
Alcaligenes sp.	–	+	–	+	+	+	+	+
Hahnia alvie	–	–	+	+	–	–	–	–

1 = Mooi river
2 = Bushmans river
3 = Mahai/Tugela river systems
4 = Mlambonjwa river
5 = Umzimouthi river
6 = Ingwagwana river
7 = Polela river
8 = Umzimkulu river

found. This bacterium was also isolated (Table 2) from rainbow trout in the Umzimkulu, Polela, Mahai and Tugela river systems. Positive IFAT results were also obtained from these rivers (Table 1). The incidences in the Umzimkulu and Polela rivers were similar to those found in the Bushmans river, but the incidence in the Mahai/Tugela river systems was very high, particularly in the hatchery at Royal Natal National Park [40 % for culturing (Table 2) and 10 % and 15 % for IFAT (Table 1)].

A. salmonicida was isolated from fish from five of the eight river systems tested. An interesting situation was noted in the Ingwagwana river where 19 % (Site 32 of Table 2) of the hatchery fish and 10 % (Site 29 of Table 2) of the non-salmonids in the river were found to be carrying *A. salmonicida*. None of the salmonids in the river were positive. It is not known whether the non-salmonids in the river act as a reservoir for the bacterium, or if these fish are carrying the bacterium as a result of high incidences in the hatchery. As *A. salmonicida* has been isolated from so many sites in Natal, the hatchery fish were probably infected by the river fish.

A. hydrophila was isolated from all of the rivers tested. The incidences of fish carrying *A. hydrophila* were generally very high, with the highest being 100 % (Table 2) from fish in the Polela river. The average incidence on all the sites was found to be 43,5 %. This bacterium is thus widespread throughout Natal. The effects of this bacterium on the health of fish in this country needs further investigation.

F. columnaris has been detected on fish from seven out of the eight rivers tested. This bacterium appears to be part of the natural microflora of the rivers in Natal.

The most serious pathogen of trout in the Transvaal, and to a lesser extent the Cape, is the trout pathogenic *Streptococcus* sp. This bacterium was not isolated from any of the sites in Natal. There has also never been any reported cases of streptococcosis in Natal. It would thus appear that this bacterium does not occur in the rivers of Natal. This finding corresponds well to the theory that the freshwater leech *(Batracobdelloides tricarinata)* is a reservoir for this bacterium (Bragg, Oosthuizen & Lordan, 1989). This leech has not been found in the rivers in the Drakensberg area but has been isolated in various rivers in the Transvaal and Cape (Oosthuizen, 1989).

Edwardsiella tarda has been isolated from fish (non-salmonids) in the Transvaal (Bragg, 1988b) but has never been isolated from fish in Natal. This bacterium was only isolated from one river system in Natal (Mlambonjwa river) (Table 2). The significance of this isolate needs further investigation.

A bacterium which was identified as *P. pisicida* according to Austin & Austin (1987) was isolated from 5 of the 8 river systems tested. The validity of the taxonomic existance of this isolate is still questionable and *"P. pisicida"* is not mentioned in Bergey's manual (Austin & Austin, 1987). The isolation of a bacterium resembling *P. pisicida* was most surprising. This bacterium is regarded as a serious pathogen of yellowtail in Japan (Austin & Austin, 1987). The pathogenicity for trout has not been recorded and there has never been a report on this bacterium causing disease in farmed trout or other salmonids. The incidences of this bacterium in the river systems of Natal were generally very high with the highest being 45 % in the Polela river. The significance of these isolations is not known, but it is unlikely that this bacterium will have any effect on the health of the trout. This is the first isolation of *P. pisicida* is this country.

Renibacterium salmoninarium was not detected on any of the sites. This bacterium has never been detected anywhere else in this country.

CONCLUSION

A number of bacteria has been detected in some river systems in Natal. Some of the more serious pathogens which have been isolated include *Y. ruckeri, A. salmonicida* and *F. columnaris. R. salmoninarium* and the trout pathogenic *Streptococcus* sp. were not isolated in Natal. Potential fish pathogens, such as *E. tarda* and *P. pisicida* were also isolated, the latter from a large number of fish. The significance of these isolations need further investigation.

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