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OUTBREAKS OF *YERSINIA RUCKERI* IN RAINBOW TROUT IN NORTH WEST OF SPAIN TITLE

L.A. RODRÍGUEZ, A. CASTILLO, C. S. GALLARDO & T. P. NIETO.
Departamento de Biología Funcional y Ciencias de la Salud, Facultad de Ciencias, Campus de Orense, Universidad de Vigo, As Lagoas, 32004, Orense (Spain)

Abstract

Yersinia ruckeri, which causes enteric red mouth (ERM) disease ,was isolated for the first time from a fish-farm in Galicia (Spain) during an outbreak in October of 1995. The identity of the isolate was confirmed as *Y. ruckeri*, by biochemical test and the properties were similar to those isolates cultured from farmed rainbow trout in Spain and Portugal.

Introduction

Yersinia ruckeri, the causative agent of enteric red mouth in rainbow trout has been isolated from rainbow trout in the U.S.A (Bullock et al., 1978), Canada (Wobeser, 1973), Australia (Llewellyn, 1980), Europe (Lesel et al., 1983; Dalsgaard et al., 1984), Spain and Galicia (Toranzo, 1990;Romalde, 1992), (Furones, 1993) .

The bacteria has been isolated from numerous diseased salmonids but is evidently capable of infecting a wide range of fish and inflicting significant mortalities (Busch, 1982). Its pathogenic capacity has been experimentally proved in rainbow trout reared in seawater and in reared marine fish such as turbot, sole, gilt-head (Vigneulle, 1984).

The present work related the first outbreak of *Y. ruckeri*, affecting a fish farming of rainbow trout in Galicia (Spain).

This bacterium has been isolated from a disease fish.

Materials and Methods.

Over a period of 6 months (September to February 1995), we sample one freshwater fish farm.. This farm is located on a small river, and fish (rainbow trout, *Oncorhynchus mykiss*) are reared in concrete tank. Samples were taken an trout farm. Each sample consisted of fish from two tanks (5-7 fish per tank).

On October 12 th 1995, unusual mortalities were observed in a single tank of the farm. Mortalities affected the biggest fish (50 g). The symptoms shown by the fish included bilateral exophthalmos, often with a whitening of the eye, a darkening of the skin pigmentation and mild submucosal haemorrhage in the mouth and under the tongue. Internally the spleen was found to be enlarged and there were severe haemorrhages in the body fat, pancreas, pyloric caeca and intestine. The liver was a bright red colour and slightly enlarged. The intestine was filled with a yellowish fluid. Similar results were obtained for other authors (Bragg & Menton, 1986)

Samples of the spleen, liver, kidney and intestine were used for bacteriology. Almost pure cultures were obtained from all of the organs sampled.

An enterobacterium, *Yersinia ruckeri*, was isolated in pure culture on Triptycase soya agar (TSA) and Shotts-Waltman (SW) (Waltman & Shotts, 1984; Hastings & Bruno, 1985) medium for differential isolation of *Yersinia ruckeri*. All plates were incubated for 48-72 h at 22-25ºC. Representative colonies of the different morphological types of the bacterial heterotrophic community, as well as the sucrose negative and oxidase negative colonies that precipitated Tween 80 (presumptive *Y. Ruckeri* in SW), were isolated.

Pure cultures of the bacteria isolated were subjected to stándar morphological, physiological and biochemical test (Busch, 1983; Romalde, 1992; Austin & Austin, 1993). The result shown in table 1.The comercial API-20E system (Biomerieux) was also used in parallel and the results were scored after 48h at 22-25ºC. Drug resistance patterns of the isolates were determined by the disc diffusion method on Müller-Hinton agar (MHA) (Difco) using the chemoterapeutic agents.

A pure culture of the isolate was used to inject twelve 100 g rainbow trout. Four 100 g trout were left as negative control fish. The infected fish began showing similar symptoms to those shown by the original infections 4 days after the injection. Pure cultures of *Y ruckeri* were isolated from the spleen, liver and Kidney of the infected fish. The biochemical test were the same as those described above.

This reisolate was used for pathogenicity assays. These were conducted at 18ºC-20ºC by intraperitoneal inoculation of fingerling rainbow trout as previously described (Nieto et al., 1985). The degree of virulence, expressed as LD_{50} (50% mean lethal dose) was calculated by the Reed and Müench method (1938).

Results and Discussion

Although *Y. Ruckeri* has been detected in most European countries (Busch, 1983; Toranzo et al., 1990; Romalde, 1992; Furones et al., 1992; Stevenson et al., 1993) until now it had not been detected in Orense (North of Spain) as a causative agent of outbreak disease.

However, the microbiological survey reported here allowed us to isolate and characteristics the Enteric Red mouth (ERM) bacterium in Orense for the first time on trout farm studied.

The culture was found to be non haemolytic on the bovine blood tryptose agar. It was found to be a gram negative rod, which was catalase positive and oxidase negative. It fermented glucose, maltose, and maltose without forming gas. Lactose, inositol, sucrose, sorbitol, arabinose and melibiose were not fermented. Nitrate was changed to nitrite but urease, indole, phenyl alaninne, H_2S production and Voges proskauer were all negative. Simmons citrate was negative. The culture was non-motile at 37ºC but motile at 22ºC. Result are shown in table I.

These biochemical reactions are similar to those of *Y. ruckeri* as described by Ewing et al. (1978).

It was found that isolate of *Y. ruckeri* was sensitive to ampicillin, tetracycline, chloramphenicol, ofloxacine and fosfomicine, while being resistant to streptomycin, Novobiocine and Gentamicine. The serotype of *Y. ruckeri* isolates was not determined although the hydrolisis of gelatine negative and sorbitol negative colonies that precipited Tween 80 presupposed a serotype O1.

The fish pathogens *Y. ruckeri* showed a DL_{50} of $3.7 \cdot 10^4$. Similar results were obtained for other authors.(Toranzo & Barja, 1993). *Yersinia ruckeri* was considered virulent strain from these results.

Table 1. Biochemical characters of *Yersinia ruckeri* strain.

CHARACTERS	
Gram	-
Motility 22ºC	+
Motility 37ºC	-
Oxidase	-
Catalase	+
O/F	F
ONPG	+
Hydrolysis of Arginine	-
Lysine */ Ornithine	+
Citrate utilisation *	-
Nitrate reduction	+
H_2S production	-
Urease	-
TDA	-
Indole	-
Voges-Proskauer*	-
Hydrolysis of Gelatin*	+
Acid from:	
Glucose	+
Mannitol	+
Inositol	-
Sorbitol	-
Sucrose	-
Maltose	+
Arabinose	-
Lactose	-
Melibiose	-

*Test exhibiting false positive or negative reactions in the API-20E System

F, fermentative.

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