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BIOCHEMICAL AND SEROLOGICAL CHARACTERISTICS OF YERSINIA RUCKERI ISOLATES WHICH CAUSE TYPICAL AND ATYPICAL INFECTIONS IN CALIFORNIAN TROUT (ONCHORHYNCHUS MYKISS)

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We were able to identify Yersinia ruckeri in 13 cases in the course of testing more than 1000 specimens from trout ponds at different locations over the period from 1978 to 1995.

The usual methods of bacterial examination (microscopic, cultural, biochemical, i. e., API 20 E, and serological) were employed.

Sorbitol fermentation was used as the biochemical characteristic to differentiate between serotypes. On the basis of agglutination with anti Yersinia ruckeri serum for serotype I (HI 70) and anti Yersinia ruckeri serum for serotype II (BC74), we were able to establish the serotypic affiliation of our isolated strains. Nine of the 13 isolated strains were found to belong to serotype I (HI 70) with regard to antigen structure, while four belonged to serotype II (BC 74).

Since Yersinia ruckeri occurs as several serotypes, it is important to stress the finding of class I serotypes (HI 70) under Yugoslav conditions. This highly virulent strain can be used for preparation of vaccines and it is applied in immunoprophylaxis.

Key words: Yersinia ruckeri, isolates, californian trout

INTRODUCTION

Enteric redmouth disease is caused by the gram-negative motile bacterium Yersinia ruckeri. The first work on the pathology and isolation of these bacteria from trout was by Ross and Rucker (1966). The bacteria were described and named by Ewing et al. (1978). Yersiniosis is an acute, subacute and chronic disease that received its name from the characteristics discernible on the mouth and operculum caused by subcutaneous hemorrhages. The disease is of economic significance, especially at certain locations with dense populations of salmonids (Mc-Daniel, 1971, Wobeser 1973). Up until now, the disease has been treated with antibiotics and through improved sanitary measures in fish ponds. Due tothe appearance of resistant strains of bacteria and limitation in connection with the use of new hemotherapeutics, the use of bacterin in prophylactic immunization has been initated for the control of fish diseases (Ross et al., 1966,

Anderson and Ross, 1972, Anderson and Nelson, 1974). Although different isolates of the bacteria are phenotypically relatively homogeneous, serological differences exist, with five serotypes currently recognisable through the use of rabbit antisera. (O 'Leary, 1977, Bullock et al., 1978, Stevenson and Airdrie, 1984). They are designated: type I HL 70 (Hageman), which is the most frequent and most virulent (Busch 1981, type II BC 74 (O'Leary) which is relatively virulent; type III (Australian) which is avirulent, and serotypes IV and V (Stevenson and Airdrie,1984;Daly et al., 1986). The virulence of serovar IV is unknown, while that of serovar V is very low. Serological characteristics of bacteria depend on antigenic structures on the cell surface. The largest surface molecules of gram-positive bacteria are lipopolysaccharides (LPS). Flett and Stevenson (1978) analysed the electrophoretic properfies of LPS from representative serotypes I, II, III and IV. Similaritiesexist between serotypes I, III, and V, and between serotypes I and II, but the latter were different from strains of serovar V. The LPS serotype II strains was heterogenous, which suggests that the serological nature of this group must depend on cross reaction between other LPS of the antigen. Serotypes as well. Although the bacteria occur in several serotypes, serotype I is highly virulent, and vaccine based on on this serotype is very protective for other serotypes as well. The purpose of our investigation was to test the biochemical and serological properties of 13 isolates of Yersinia ruckeri from Yugoslavia.

MATERIAL AND METHODS

Selection of isolates. The isolates tested in the present work are listed in table 1. They all had the characteristics of Yersinia ruckeri and are being stored in pure culture on brain-heart infusion agar.In addition to our isolates American strains of serotype I (HI 70) and serotype II (BC 74), obtained through the kindness of Prof. Rohovec of Corvallis, Oregon (USA), were used.

Our isolates and the American strains of Yersinia ruckeri were preliminarily subjected to ciochemical testing on the API 20 E system for enterobacteria.

All 13 isolates were taken for serological comparison by the method of rapid agglutination on a microscope slide with a known antiserum to Yersinia ruckeri. Antisera to Yersinia ruckeri were prepared from the American strains, namely from serotypes HI 70 and BC 74.

Antigens for immunisation. Strains I and II of Yersinia ruckeri for immunisation were cultivated for 48 h in soyabean bullion tryptose, and the bacteria were inactivate by addition of 0,4% formalin (35%) to the culture. The cells were then collected by centrifugation and rinsed twice in physiological saline.

Preparation of antiserum Rabbits were intramuscularly injected in the hind flank with a suspension (in physiological saline) of formalin-inactivated cells (10 log 9 cells/ml, Mc Farland standard No. 3).

The injections were given in individual doses. The rabbits were intramuscularly inoculated five times at three day intervals. For inoculation I we took 0,5 ml of antigen with 0,5 ml of Freund's complete adjuvant. After three days,inoculation II was performed using 0,5 ml of antigen without the adjuvant. Antigen was

taken in the quantity of 1 ml for inoculation III, while 2 ml of antigen was administered in both inoculations IV and V. Antiserum was prepared 3 weeks later and stored at -20^{0}C until use.

Plate agglutination tests. One batch of bacterial cells that grew on TSA was added to 0,5 ml of PBS and mixed to obtain a homogeneous suspension. One drop of each of three ingredients-PBS, bacterial cell suspension, and antiserum was added to one depression of a concave plate. Two drops of PBS and one drop of bacterial cell suspension were introduced into the other depression as a control. The plate was carefully rotated for 2 min. Agglutination of cells was determined macroscopically and microscopically.

RESULTS AND DISCUSSION

Thirteen isolates of Yersinia ruckeri were obtained from different localities in the course of research over a period of years on the territory of Yugoslavia (Table 1). Biochemical testing and comparison with American serotypes I and II (HI 7O and BC74) confirmed that these isolates differed among each other (Table 2). The biochemical characteristics of our 13 isolates and the American serotypes I and II produced beta galactosidase, lysine decarboxylase, and ornithine decarboxylase. They fermented glucose and mannitol. Serotype II also fermented sorbitol, which differentiated it from serotype I. The other biochemical reactions were negative.

Table 1. The cultures of Yersinia ruckeri isolated from californian trout which were used in the examination

Number	Location	Date of isolation
2283	Žalec	1987
509	Žagubica	1989
1689	Despotovac	1990
541	Žagubica	1990
950	Konjic	1991
2116	Sisevac	1991
2443	Podgorica	1991
3065	Novi Pazar	1991
1437	Nova Varoš	1992
1774	Istok	1993
693	Sjenica	1994
1673	Berane	1994
214	Istok	1995

The Yersinia ruckeri isolates designated by the numbers 950, 2443, 1437, and 214 reacted like serotype II BC 74, that is they fermented sorbitol.

Table 2. Biochemical characteristic of 13 isolates of Yersinia ruckeri and two American serotypes (HI 70 and BC 74) (after incubation at 20°C for 48h)

	HI 70	BC 74	2283	509	1689	541	950	2116	2443	3065	1437	1774	693	1673	214
ONPG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADH	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LDC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ODC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CITRAT	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
H_2S	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
UREA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TDA	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
INDOL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
VP	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
GELATIN	–	–	+	+	–	+	–	–	–	+	–	–	+	+	–
FERMENTATION	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GLUCOSE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MANNITOL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
INOSITOL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SORBITOL	–	+	–	–	–	–	+	–	+	–	+	–	–	–	+
RHAMNOSE	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SALICIN	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
MELEBIOSE	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AMYGDALIN	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AMYGDALIN	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
REDUC. NO_2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The other isolates did not ferment sorbitol, but differed from serotype I HI 70 in regard to gelatin decomposition. This group included Yersinia ruckeri isolates nos. 541, 509, 3065, 693, and 1673. Isolate 2283 differed biochemically from serotype I HI 70 by breaking down gelatin and in having a positive Voges-Proskauer reaction.

The serological reactions were used to diagnose and identify Yersinia ruckeri, the agent of enteric redmouth disease. The serological reactions mentioned herein ars used in the majority of laboratories.

Thirteen cultures of Yersinia ruckeri were tested by the method of agglutination on a microscope slide using the prepared antisera to Yersinia ruckeri HI 70 and BC 74. Agglutination was complete with all cells bound together, and was macroscopically discernible (Table 3).

Table 3. The results of the coverslide agglutination test between 13 isolates of Yersinia ruckeri and antisera prepared against serotypes HI 70 and BC 74

Antigen	Antisera		Serotype
	HI 70 (1)	BC 74 (2)	
HI 70	+	–	1
BC 74	–	+	2
2283	+	–	1
509	+	–	1
1689	+	–	1
541	+	–	1
950	–	+	2
2116	+	–	1
2443	–	+	2
3065	+	–	1
1436	–	+	2
1774	+	–	1
693	+	–	1
1673	+	–	1
214	–	+	2

+ positive reaction
- negative reaction

It can be seen from the obtained results that both serotypes of Yersinia ruckeri exist in Yugoslavia.

Nine cultures of Yersinia ruckeri (2283, 509, 1689, 541, 2116, 3065, 1774, 693 and 1673) belonged to serotype I (HI 70),while four cultures belonged to serotype II (BC 74) of Yersinia ruckeri.

Other investigators found that cultures of Yersinia ruckeri either do not ferment sorbitol (Ross et al. 1966; Bullock and Sniesyko, 1975; Jeremić and Perić 1991) or else ferment it weakly (Ewing et al. 1978; Stevenson and Daly 1982).

Of the 13 cultures listed in table 2, four (950, 2443, 1437 and 214) fermented sorbitol and were serologically identified as type II (BC 74) Yersinia ruckeri, that is they were sorbitol positive, whereas the nine isolates of serotype I (HI70) were sorbitol-negative. In the course of study over a period of years, we demonstrated the existence of Yersinia ruckeri serotypes I and II in populations of diseased trout in different localities.

Although Yersinia ruckeri occurs in several serotypes,serotype I is highly virulent, and vaccine based on this serotype is very protective for other serotypes as well.

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BIOHEMIJSKE I SEROLOŠKE KARAKTERISTIKE IZOLATA YERSINIE RUCKERI KOJI SU IZAZVALI TIPIČNU I ATIPIČNU INFEKCIJU KOD KALIFORNIJSKE PASTRMKE *(ONCHORHYNCHUS MYKISS)*

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SADRŽAJ

U toku višegodišnjeg ispitivanja od 1978. do 1995. godine, kojima su obuhvaćeni različiti lokaliteti pastrmskih ribnjaka sa preko 1000 materijala uspeli smo da identifikujemo u 13 slučajeva Yersiniu ruckeri.

U ispitivanjima smo koristili uobičajene metode bakterijskog pregleda (mikroskopski, kulturelno, biohemijski - API 20 E i serološki).

Od biohemijskih osobina u cilju diferencijacije serotipova koristili smo fermentaciju sorbitola.

Na osnovu aglutinacije sa anti Yersinia ruckeri serumom serotip I (HI 70) i anti Yerskinia ruckeri serumom serotip II (BC 74) uspeli smo da ustanovimo serotipsku pripadnost naših izolovanih sojeva. Od 13 izolovanih sojeva ustanovili smo da 9 pripada po antigenoj strukturi serotipu I (HI 70), koji su izazvali tipičnu kliničku sliku jersinioze. Ribe su pokazivale krvarenja u zoni usne duplje, nepca, jezika, jugularne regije, operkuluma i perifaringealne regije.

Preostala 4 soja pripadaju serotipu II (BC 74) koji su izazvali atipičnu infekciju bez karakterističnih znakova jersinioze.

Pošto se Yersinia ruckeri javlja u nekoliko serotipova značajno je istaći nalaz serotipa (HI 70) u našim uslovima. Ovaj visoko virulentni soj može se koristiti za pripremanje vakcine i njene primene u imunoprofilaksi.