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Identification and treatment of diseases in the common sole (Solea solea L.)

by

J.Flüchter
Bayerische Landesanstalt für Fischerei
Starnberg

Inducement to the following investigations was a permanent endangering of long term experiments, especially of the attempts to rear soles in captivity.

Long term perliminary work was repeatedly nullified by the extermination of the stock of adapted soles by epidemics and parasistical plagues. The development of methods to combat this calamities and to find out pinpointed prophylaxis was therefore a prerequisite for continuation of the rearing experiments in sole. Observation of soles for several years enabled to discriminate between three main diseased states which occured repeatedly:

1.) An irritation starting at the edge of the caudal fin and progressing forward to the integument and muscle tissue. In advanced stages the caudal fin was lost and spines of the body skeleton became visible. At this stage, about two or three weeks after the first irritation occured, the fish generally died. Similar symptoms were described for several other species by other authors and named tailrot.

2.) Different from 1.) soles developed several swellings about 10 mm in dameter on both sides which burst to open furuncles. So it may be succinctly named furunculosis. The soles survived with the abscesses for more than a month and sometimes the furuncles healed up spontaneously when the fish was transferred to unpolluted water early enough.

3.) While tailrot and furunculosis are slowly progressing and are to identify early enough for treatment, there is a disease, which is poor in symptoms, showing only a haemorrhagic reddish miscoloration of the blind side and pale gills, this symptoms became visible not until two days before death. It may succinctly be named redspot disease. For this plague was very contagious and rapidly progressing, it was the main endangering of stocks of adapted soles. The mortality of adapted soles caused by diseases came to catastrophic amount when I had to share with a guest colleague, who kept many dabs in the same, without that to small, closed water-circulation. The organic pollution of the water by the wastes of the dabs which are fishfeeders, obviously supported the outbreak of diseases.

As first step to bring this diseases under control, a bacteriological investigation seemed to be promising. For the bacterial inquiry firstly the skin was sterilized by ethanol and burning. Then the body cavity was opened under sterile conditions and a smear was taken with a platinum wire. As the next the pericardium was opened and blood was taken sterile from the sinus venosus. Later on kidney was included in this procedure. In the same manner from musculature and from the connective tissue between musculature and skin smears were taken. As culture medium a simple seawater nutrient agar, developed for culture of non pathogenic marine bacteria was used (Tab.1)
It was poured warm and liquid into petridishes 9 cm in diameter 15 ml each and allowed to solidify. Then the smears were streaked on the surface of the medium.

Firstly adapted fishes with the symptoms were investigated. Indeed always many colonies developed when the smears were taken from body cavity, from blood and from kindney. No colonies developed when smears were taken from the musculature or from connective tissue.
The type of bacterial colonies was different belonging to the disease. The moderate nutrient claims of this pathogenic bacteria should be stressed. Upon the given culture agar tailrot and furunculosis developed creamcoloured colonies about 2 mm in diameter, while " red spot disease" was characterized by small (ca. 0,5 mm Ø), clear colonies but with an acrid suppurative smell. Tailrot bacteria were determinated as belonging to the Pseudomonas group, exitant of " red spot disease" was identified as near relative to Vibrio anguillarum by Prof. Shewan Aberdeen as specialist. Much bigger colonies were obtained when an extract of tryptic digested fish tissue was added. But to standardize this homespun culture-medium needs a too high expenditure.
To get imagination about bacterial infection in the natural environment, soles freshly caught at sea and external looking healthy were included in the investigation. It was surprising that about 80 % of these soles were latent infected. Evidence for isolated bacteria to be indeed the moribific agent is the reproduction of the disease by reinoculation of an in vitro culture to healthy soles in this case. This evidence could be produced with certainty in case of red spot vibrios.
Artificial infected soles certainly died within four days after infection but the typical symptoms became visible two days or only one day before exitus. This vibrios, stored for one year on nutrient agar were

full infective. That indicates the special danger conjured up by this excitant.

For experiments to bring this contagious diseases under control by using antibiotics, in vitro methods must be prefered, for adult adapted soles were too precious for broadspread contagion experiments with living fishes. For testing to which antibiotics the pathogenic bacteria are sensitive, it was made use of the agar diffusion test. To find out the sensitivity of the pathogenic germs to antibiotics, so called sensitivity disks and stars were used, sterile paper disks containing known amounts of antibiotics. A single colony was uniformly distributed upon an agarplate, then the sensitivity disk or star was put on and then incubated at 20°C, later on 25°C for the multiplication of the bacteria was more rapid at the higher temperature and so results could be earlier obtained. The antibiotics diffuse into the nutrient agar and there zones where susceptible bacteria do not grow become visible.

In this way the susceptibility of the pathogenic germs to the different antibiotics could be determined. This test allowed furthermore, basing upon the specific sensitivity of the different pathogenic bacteria to antibiotics a very rough but very rapid and for this purpose sufficient classification. It should be insisted that susceptibility to Benicillin as furunculosis shows is rarely occuring in marine bacteria generally and that red spot disease vibrio is not sensitive to Colistin while tailrot is sensitive to Colistin but resistent to Penicillin (see tab.2). The excitants of the diseases described here are all of sufficient susceptibility to Chloromycetin (tab.2). But Chloromycetin could not be injected, for it is of insufficient watersolubility.

For oral application the rapid sedimentation hindered to dose exactly. When Chloromycetin was suspended in 1 % carboxy-methyl-cellulose-water-solution, the suspension was stable enough for exact dosage. While a medical treatment of furunculosis and tailrot by application of Chloromycetin was successful, the red spot vibrio disease was not treatable in practise even by in vitro successful antibiotics. The rapid progress of this disease and the late appearance of the symptoms hindered control by antibiotics. Application of Chloromycetin to soles following vibrio infection even forced the exitus of the fishes.The virulence of this vibrios enabled them, not only to infect feable soles but as well as excellent conditioned ones.

Basing upon the ability to survive for a long time and the capability

of rapid multiplication on organic wastes as nutrient matter -as tested in the experiments described above- the red spot disease vibrios were a steady menace to the experiments in such a degree that pinpointed prophylactic actions were absolutely indispensible.

As a way out of this difficulty artificial immunization was tried. To make specific defensive reactions active against virulent pathogenic bacteria, the injection of those pathogenic germs but inactivated before, is a proved method since many years.

One of the methods to inactivate pathogenic bacteria tenderly, is to heat them up to 56°C for one hour.

To use a measure which can be reproduced, in practise the bacterial mass which overgrow a nutrient-agar plate (about 60 cm^2) -as dose for one fish- was scraped off, suspended in 1 ml physiological NaCl -watersolution, heated as described and at last this so called vaccine was injected intramuscular. After vaccination the soles did not feed and were very indolent for one week. Two weeks after vaccination, soles became infected by injection of a high dose of living vibrios. Each fish received as dosage the upgrowth of half a petri plate (about 30 cm^2) suspended in 1 ml seawater.

Though such a massive infection never occured in the natural environment, no one of the ten vaccinated soles died after artificial infection.

Two times five invaccinated soles each were infected as a control. They all died within four days after injection. The application of Chloromycetin may be of practical value in tagging experiments, to avoid secondary infections of the injuries. Soles survived even severe wounds when screened against bacterial contagions.

Observations lead to the suspicion that zooparasites would be pacemakers for diseases. By perforating the skin of the soles they enabled the pathogenic bacteria to invade the fishes. While the extermination of parasitic crustaceans and leeches was possible by using a bath of 2,2,2-trichlor-1-hydroxy-athylphosphoneacid-dimethylester (Masoten Bayer) 25 g/litre for five minutes, there remained an ectoparasitic trematode Entobdella solea which was resistant to all usual chemical treatments. Mechanical control was not practicable in often occuring cases of massive attacks by this parasite. At least as way out of this calamity a special warmwater treatment was found to be successful in fighting this parasite. When the temperature successively was raised (3°C per day) to 30°C as upper limit, the trematodes disappeared

while soles survived this treatment.
The description of symptoms of diseases and methods to control bacterial and parasitical disasters may be a step in direction of screening long term experiments on fishes against biological disturbances.

Table 1

Composition of culture medium
(Zobell 2216 E)

5	g	bacto peptone
5	g	bacto yeastextract
15	g	bacto agar
0,01	g	$FePO_4 \cdot 4H_2O$
750	ml	aged seawater
250	ml	aqua dest
		Ph 7,6

Table 2

Sensitivity of the pathogenic bacteria to some antibiotics

	tailrot	furunculosis	red spot disease
Penicillin	(-)	+ +	(-)
Dihydrostreptomycin	+	+	(+)
Ampicillin	(+)	+ +	(-)
Tetracyclin	(-)	(+)	(-)
Chloramphenical *	+ +	+ +	+ +
Spiramycin	(-)	+	(-)
Kanamycin	(+)	+	(-)
Colistin	+ +	+ +	(-)

(-) resistant
\+ sensitive
(+) sensitive but not all strains
\+ + good sensitive
*Chloramphenical = Chloromycetin