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ON THE INTERNAL BACTERIAL FLORA OF THE EUROPEAN LOBSTER, HOMARUS VULGARIS L., AND ITS SUSCEPTIBILITY OF GAFFKAEMIA

by

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Abstract.

The internal microcbccal flora of 23 healthy European lobsters, Homarus vulgaris L., was investigated. The lobster pathogen, Gaffkya homari, was not found. A new solid medium giving a more abundant growth of the bacterium is described. The susceptibility of G. homari for the European lobster with A.T.C.C. type strain no. 10400 was tested. By injecting this strain into healthy lobsters a mortality of 100 % was obtained. The occurence of the bacterium in Norwegian waters is discussed.

Introduction.

In 1955 and more seriously in 1957 Dutch lobster importers suffered heavy losses due to the infectious disease Gaffkaemia (Roskam, 1957). The agent of the disease is a tetrad-forming micrococcus, Gaffkya homari, first described by Snieszko and Taylor (1947) and named by Hitchner and Snieszko (1947). The bacterium causes a septicemia, resulting in loss of circulating blood-corpuscules and increased clotting time. The disease is highly infectious in pounds and tanks. The bacterium is found in pure culture in the body fluids of the dead animals. The disease seems to be dependent on temperature, occuring in summer only at water temperatures above 8° C.

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Dutch workers (Roskam 1957 and 1958) report the disease in lobsters imported from Norway in May and June 1957 and in 3 dead lobsters in 1958.

In Norway however, the disease has never been reported and the aim of this work was to investigate the bacterial flora of the internal organs of the European lobster, Homarus vulgaris L., to ascertain wether G. homari might be part of the natural bacterial flora of the lobster causing disease only under suitable conditions. Pathogenicity tests of G. homari, for the European lobster, were also performed.

Experiments and results.

The internal organs of 23 healthy lobsters from the pound at Glesvær south-east of Bergen, were examined. Several different methods of killing the animals were tried, and the easiest was found to be to deep-freeze the animals and then to disect out the internal organs under sterile conditions. Cultures on several culture media. were made from heart, liver, green glands, stomach, intestine and reproductive tissues. Also some cultures were made from muscle. A copepod., Nicothöe astaci Adouin and Milne Edwards, which seemed to be a very common gill parasite on the lobster, was disinfected, crushed and spread on to solid culture media.

The results of the investigation showed that heart, liver, green glands, reproductive tissues and muscle seemed to be sterile. Also the cultures from the gill parasites seemed to be sterile. From the stomach and intestine on the contrary a rather varied microflora was grown consisting of different bacteria, some fungi and several yeast-like organisms. As only little is known about the microflora of marine evertebrates, it would have been of interest to make a survey of the whole microflora of the lobster, but the work had to be limited and was concentrated on the micrococcal flora. All colonies were examined by phase-contrast microscopy and all micrococcae were picked out for further examination. Here again the material had to be limited and at last 53 strains of small micrococcae that could possibly be identical to G. homari were left.

These 53 strains were determined according to the list of reactions given for G. homari in Bergey's Manual of Determinative Bacteriology (1957). The following reactions were tested: Growth in

nutrient broth and on nutrient agar, on potato, the ability to liquefy gelatin and hydrolyse starch, to reduce NO_3 to NO_2, production of indol, H_2S and acetylmethylcarbinol and the reactions on the following sugars and alcohols: glucose, lactose, saccharose, maltose, arabinose, xylose, mannitol, dulcitol, inositol, salicin and glycerin. Further the production of urease, the use of ammonium salts as sole source of nitrogen and the reaction on litmus milk were tested.

Although most of the examined micrococci did resemble G. homari in that they were little reactive, none of them were consistent with it in all reactions.

For comparrision and control the type strain G. homari, A.T.C.C. no. 10400 was obtained. The description of this bacterium says it grows slowly and sparsly on artificial media. A medium of the following composition: 0.5% peptone, 0.5% tryptone (Oxoid), 0.5% yeast extract, 0.5% NaCl, 0.1% glucose, 0.2% Na_2HPO_4, 0.5% Bacto Thiol Medium and 1.5% agar, was found to give a fairly abundant growth in 2 - 4 days at 30°C. It also appeared that the lyophilized culture was more easily grown than when the type strain was received grown in Difco Bacto Thiol Medium.

Snieszko and Taylor (1947) obtained a 100% mortality when injecting the American lobster, Homarus americanus Milne Edwards, with emulsions of the bacterium G. homari in 2.5% NaCl grown on artificial media. The Dutch investigators (Roskam, 1957) were not able to transfer the disease this way, they succeded with a 67% mortality only by transfering blood from diseased animals. This fact was believed to be explained either by the Dutch strain of G. homari to be less virulent than the American one, or European lobster to be less susceptible of the disease.

In this investigation 7 healthy lobsters from the Glesvær pounds were injected with 0.5 ml of a dense emulsion of the type strain grown on the artificial medium mentioned above, in 0.5% NaCl. The injection was made in the muscles of the first abdominal segment. The animals were held in aerated sea-water at 16 - 18°C. All animals died within 5 days. The bacterium was found in pure culture in rather large quantities in the body fluids of the dead animals and was easily reisolated. In a later similar test with 9 lobsters held at 14°C, all animals died

within 9 days, also here the bacterium was easily regained from the body fluids. In both experiments control animals held under quite similar conditions did not show any signs of disease.

Conclusion.

The work refered to in this paper was carried out in the early sixties and although there are several more recent publications on this subject, it may be of some interest that studies concerning this disease have been done in Scandinavia. The conclusion of this investigation must be that the disease Gaffkaemia has not occured to any obvious extent in Norwegian waters but that the local lobster is nevertheless highly susceptible to the pathogen. Although G.homari was not detected in the normal flora of the lobsters tested, the sample was too small to exclude the theory that it might be part of the natural bacterial flora of the lobster causing disease only when conditions become unfavorable. Stewart et al., 1966, found that almost 5 % of a total of 2035 apparently healthy lobsters investigated contained G. homari.

In more recent papers (Stewart et al., 1966) is refered to "epizootics in Norway" described by Roskam 1957. To our knowledge the disease Gaffkaemia has never occured in Norway, the "epizootic" in the Dutch paper refers to the bacterium being found in two lobsters consignments said to come from the Norwegian west coast.

According to Roskam (pers. com. 1958) imported lobsters are stored in series of shallow tanks at Yerseke in the Rhine-Schelde estuary and usually his laboratory received samples for examination by mail with only the importers word as to where the cases originated. To our knowledge investigations of the possible presence of Gaffkya in the storing tanks and the surrounding waters at Yerseke, have not been made, and occurence of the disease due to infection in the tanks etc. can not be totally excluded, as Wood (1965) has shown that the disease can be transfered by infected water.

Inquiries made to several Norwegian lobster pounds and of the Lobster Exporters Association (Hummereksportørenes noteringsutvalg) in 1960 and again last year, gave no evidence at all of any infectious disease having occured among the local lobster populations in Norwegian waters. The temperature at the lobster pound of Glesvær near Bergen and around Kvitsøy near Stavanger may reach 15 - 17° C during summer.

It must be added that from time to time there are imports of lobsters from Scotland to western Norway for later re-exportation to the continent. According to Roskam (1957) the disease in Holland was first reported in a consignment said to originate from Scotland. To our knowledge the disease is not reported in Scottish waters. Further investigation as to where the organism originates in Europe should be encouraged.

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