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AEROMONAS SALMONICIDA A POTENTIAL PATHOGEN IN MODERN EEL (*ANGUILLA ANGUILLA*) FARMING?

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Abstract

A fish farm producing turbot suffered from serious and persistent disease outbreaks caused by *A. salmonicida* subsp. *salmonicida*. The farm wanted to start an intensive production of eels, too, but were concerned about the potential risk of infections with *A. salmonicida* subsp. *salmonicida*. Our laboratory carried out a series of pathogenicity tests on eels to evaluate the risk. By immersion of eels in bacterial suspensions with densities up to $1.1x10^{10}$ c.f.u./ml, no mortality was induced and no clinical or pathological changes were found. By injection of the bacteria i.p. or i.m. mortalities were recorded but only at high doses of bacteria. Thus, LD_{50} values of $7.4x10^{7}$ and $5.0x10^{8}$ c.f.u. were calculated for the i.p. and the i.m. routes, respectively. We therefore conclude that although eels were susceptible to high doses of *A. salmonicida* subsp. *salmonicida*, the risk of infections in an eel farm must be considered to be minimal under normal circumstances.

Introduction

Aeromonas salmonicida subsp. *salmonicida* is mainly a pathogen for salmonid fish but an increasing number of reports of infections in other fish species occur. These infections usually occur under stressful conditions or when the pressure of infection is severe, and should not be confused with infections caused by atypical *A. salmonicida*, which seem to occur in almost any fish species. Infections by atypical *A. salmonicida* have also been described in eels (Pedersen *et al.* 1996). In addition to salmonids, the typical *A. salmonicida* subsp. *salmonicida* has been associated with disease in at least cod, halibut, wrasse, and turbot (Hjeltnes *et al.* 1995, Nougayrede *et al.* 1990, Pedersen & Larsen 1996, Toranzo & Barja 1992, Treasurer & Cox 1991, Treasurer & Laidler 1994), and it has been isolated from healthy fish of various species (Samuelsen *et al.* 1992, Willumsen 1990) as well as from water and sediment near net cages (Enger & Thorsen 1992) and marine zooplankton (Nese & Enger 1993). Experimental infections have revealed that *A. salmonicida* subsp. *salmonicida* was pathogenic to turbot and halibut larvae (Bergh *et al.* 1997). Recently, we described a serious and prolonged outbreak of furunculosis in a Danish turbot farm (Pedersen & Larsen 1996) with high mortalities over several months. The same farm had previously cultured rainbow trout, which were assumed to be the source of the infection although there had not been rainbow trout on the farm for about two years at the time of onset of the disease problems. This fish farm recently started an intensive production of European eel (*Anguilla anguilla*), and since the *A. salmonicida* subsp. *salmonicida* obviously was extremely persistent on the farm and capable of causing serious outbreaks of disease in the atypical host, turbot, the owners of the farm were concerned about the potential risk of similar outbreaks in eel. Therefore, our laboratory was requested to carry out a virulence study to evaluate the potential virulence of *A. salmonicida* subsp. *salmonicida* to eels. The present paper describes the results of our challenge studies on eels using intramuscular (i.m.) or intraperitoneal (i.p.) injection of bacteria or immersion.

Materials and methods

Bacterial strains and culture conditions.

A virulent strain of *A. salmonicida* subsp. *salmonicida*, isolated from diseased turbot (Pedersen & Larsen 1996) was used for the study. The bacteria were propagated in brain heart infusion (BHI) broth (Difco) in 1 l Erlenmeyer flasks incubated under vigorous agitation overnight at 20°C, centrifuged, and resuspended in volumes of phosphate buffered saline (PBS, pH 7.3) as necessary to obtain adequate cell densities. Ten-fold dilu-

tions were made in PBS and plated on tryptic soya agar (TSA) (Oxoid) for making viable counts.

Fish. Eels, 15 - 20 g, were kept in 20 l plastic tanks in freshwater at room temperature, approximately 20-22°C, under constant aeration. The water was changed daily. During the experiments, the eels were not fed and they received no medication or other treatment, except that the eels injected i.p. or i.m. were anaesthetised with MS-222 prior to injection.

Virulence experiments. Initial experiments using ten-fold dilutions were carried out to estimate the approximate lethal doses by i.p. and i.m. injection, respectively. On the basis of the results of these tests, a set of three- or ten-fold dilutions were made within a more narrow interval of concentrations of bacteria, in order to determine LD_{50} values as precisely as possible. Six groups of 6 fish were injected i.p. with 0.1 ml of serial dilutions of the bacteria ranging in the interval $2.1x10^8$ - $5.2x10^{10}$ c.f.u./ml, whereas i.m. injections were made using 0.1 ml volumes of bacterial suspensions with concentrations ranging from $7.85x10^4$ to $7.85x10^9$ c.f.u./ml. The i.m. injection was done deep in the musculature approximately 1 cm behind the head.
For the immersion experiments, 6 groups of 6 fish were transferred to buckets where they were immersed for 30 min in bacterial suspensions, $4.5x10^7$-$1.1x10^{10}$ c.f.u./ml. After exposure to the bacteria the eels were transferred back to their tanks. All fish were observed repeatedly every day for up to 2 weeks. Mortality as well as clinical and pathological manifestations were recorded and LD_{50} values were then calculated by the method of Reed & Muench (1938).

Table 1. LD_{50} values for *A. salmonicida* subsp. *salmonicida* in eels infected by the immersion or i.p. or i.m. injection routes.

Infection route	LD_{50} (c.f.u.)
i.p. injection	$7.4x10^7$
i.m. injection	$5.0x10^8$
immersion	$>1.1x10^{10}$

Results
The results in terms of LD_{50} values are shown in Table 1. In the groups infected by immersion no mortality occurred and no fish displayed clinical or pathological abnormalities. In the groups of fish infected by i.p. injection, some mortality was recorded and LD_{50} was calculated to $7.4x10^7$ c.f.u. The eels developed pathological changes similar to those described for furunculosis in salmonids: haemorrhages at the site of injection, often with necrosis, haemorrhages - more or less pronounced - in the skin, particularly along the fins, around the mouth, and in the head, and a protruding anus with hyperaemia or haemorrhages. Some fish developed skin ulcers. These ulcers had the appearance like those described in turbot (Pedersen & Larsen 1996) but different from the typical furuncles found in salmonids (Bruno & Poppe 1996): they were 2 - 10 mm in diameter with a haemorrhagic centre surrounded by a whitish zone but were restricted to the dermis and superficial layers of the muscle tissue, and did not form typical furuncles with affection of dermis as well as deep musculature necrosis and liquefaction.
At autopsy, haemorrhages were present in internal organs and a serohaemorrhagic fluid was present in the abdominal cavity and intestinal lumen.
By i.m. injection a somewhat higher LD_{50} was calculated (Table 1). Dead fish displayed the same pathological changes as fish that succumbed to i.p. injection. In general, the course of the disease had a tendency to be more acute after i.p. than after i.m. injection. Most fish died 2 - 4 days after i.p. infection, whereas fish infected by the i.m. route died on day 4 or later. The pathological changes therefore tended to be more chronic after i.m. injection with skin ulcers being more pronounced. Some fish developed several ulcers distributed on the whole body surface. At the injection site, necrosis of the musculature occurred with development of a furuncle. Also many surviving fish developed pathological changes at the injection site: swelling, haemorrhages in the skin and mus-

culature, some had necrosis of skin and muscle tissue, and in some eels there was discolouration of overlying and surrounding skin.

Discussion

From the results we conclude that *A. salmonicida* subsp. *salmonicida* is in general not pathogenic to eels. From the immersion experiments it seems that the bacterium is unable to penetrate the physical barriers of the eels, and from the injection experiments it became clear that the infective dose was very high. Therefore, under natural circumstances, the bacterium will not be able to cause infection. However, under certain, stressful circumstances it can not be excluded that disease in some fish may occur, for example. if density of eels is high, and some of the fish have damages of the integument as the result of parasite infections or mechanical damages. However, we consider the problem to be minimal.

For comparison, the lethal doses of the eel pathogen *Vibrio vulnificus* biotype 2 (serogroup E) have been shown to be considerably lower. Thus, in an experiment (Amaro *et al.* 1996) where elvers were injected with *V. vulnificus* strains, the following LD_{50} values were recorded: biotype 2, opaque (encapsulated) form: $4.1x10^2$ c.f.u., translucent (non-encapsulated) form: $3.5x10^4$ c.f.u., biotype 1, opaque form: $>10^8$, opaque form: $7.8x10^4$. In another study where more strains were included (Biosca *et al.* 1993), LD_{50} values for biotype 2, encapsulated strains were $10 - 10^4$ and for non-encapsulated strains $10^4 - 10^6$ c.f.u. All these figures are much lower than the values we have found for *A. salmonicida* subsp. *salmonicida.*

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