Oral and parenteral active immunization of carp against *Aeromonas punctata*

by W. Schaperclaus

*Original title:* Orale und parenterale aktive Immunisierung von Karpfen gegen *Aeromonas punctata*

*From:* Archiv für Experimentelle Veterinarmedizin (Archive for Experimental Veterinary Medicine), 26(5) : 863-874, 1972

Translated by the Translation Bureau (MMA)
Multilingual Services Division
Department of the Secretary of State of Canada

Department of the Environment
Fisheries Research Board of Canada
Halifax Laboratory
Halifax, N. S.

1973

22 pages typescript
Oral and parenteral active immunization of carp against Aeromonas punctata.

Archiv für Experimentelle Veterinärmedizin

Reference in English - Référence en anglais:

Archive for Experimental Veterinary Medicine

Publisher - Éditeur:

S. Hirzel Verlag

Place of Publication - Lieu de publication:

Leipzig (East Germany)

Year - Année:

1972

Volume - Volume:

26

Issue No. - Numéro:

5

Number of Typed Pages - Nombre de pages dactylographiées:

22

Requesting Department - Ministère-client:

Environment

Branch or Division - Direction ou division:

Fisheries Service

Person Requesting - Demande par:

A.T. Reid - Scientific Documentation

For Dr. W.D. Paterson, Halifax, N.S.

Date of Request - Date de la demande:

October 23, 1973

Uncorrected translation

For information only

Traduction non révisée

Information seulement
Arch. F. Experimentelle Veterinärmedizin 26, #5: 863-874 (1972).
(Received 27 January 1972)

Prophylaxis by active immunization is a promising approach in bacterial fish infections generally causing substantial loss. In regard to defense against *Aeromonas punctata* (*A. punctata*) (Zimmerman, 1890, emended by Lehmann and Neumann, 1896) Snieszko comb. nov., which produces devastating 'infectious ascites' with its diverse symptoms, especially in carp, it has been known, however, that particular attention must be paid to pathogenesis when antigen vaccines are prepared (Schäperclaus, 1970). While the pathogenicity of *A. punctata* is primarily due to its exotoxic hemolysins, some endotoxic properties also play a role. Classification by pathogen-host relations places it in the widespread 'toxic-reactive' group of pathogenic bacteria.

Other factors to be borne in mind are epizootiological peculiarities. In many respects *Aeromonas punctata* infections resemble the coli enteritis of infants or pigs. Numerous types of *A. punctata* are ubiquitous in the environment and in carp feed. These bacteria are almost always present in the intestines of carp and are taken up time and again; they can therefore be regarded as part of the normal intestinal flora. But, occasionally, when the reactivity of...
defense mechanisms of carp has been altered, especially when the resistance of the fish and their condition have been weakened by hibernation, hunger, injury or otherwise impaired maintenance, bacteria invade the intestinal wall, whence they reach the circulation and organs of the abdominal cavity. If the infection takes a favorable course, antigens form and a 'basic immunity' against the partial antigens of the A. punctata types that have penetrated is established. Repeated invasions broaden the active margin of immunity and strengthen it by a type of booster effect. But, massive invasions under unfavorable conditions result in ascites. In these circumstances ordinarily harmless bacteria become potent pathogens. Thus, like Escherichia coli (Sedlak and Rische, 1968), A. punctata may be regarded as 'conditionally pathogenic'.

Their omnipresence is one of the major obstacles in preparing promising vaccines against A. punctata, another is the great number of so far barely identified serotypes and lysotypes endowed with more or less substantially different biochemical activity and resistance to chemotherapeutic agents.

The technical problems encountered in vaccinating the fish are equally formidable. Industrial fish breeding, where large numbers of fish are kept in a narrowly confined space, predictably increasingly aggravates the epizootiological situation, and requires simple 'needleless' immunization techniques. These methods should require a minimum of labor and make it possible to immunize fish stocks appropriately at an early age to achieve adequate protection against the imminent risk of infection. Naturally, parenteral injection of vaccine, used successfully in the past for two-summer carp fry are no longer feasible. Currently the only possible approach is oral application of vaccines with the feed. Inciden-
tally, carp and all cyprinids offer most favorable prerequisites for this approach: they possess no stomach where the ingested vaccines might be altered or destroyed by low pH values.

We have already demonstrated in our earlier publication mentioned above (Schäperclaus, 1970) that active immunization of carp by feeding oral vaccines is possible. Based on past results, the following questions forming the subject of our present study arose:

1. Is it possible to achieve effective active immunization by oral administration of vaccines in a large enterprise?
2. Are newly developed vaccines which - along with endotoxins - primarily contain exotoxoids of highly virulent A. punctata strains useful for this purpose?
3. How effective are such 'needleless' oral vaccination procedures, especially in industrial warm water production tanks?

Obviously, the question of testing oral immunization with streptomycin-dependent A. punctata organisms also arose in the light of the excellent results in oral immunization against coli enteritis recently published (Linde et al., 1969, 1970; Raettig and Marquardsen, 1970). But, as the number of such experiments carried out to date is still too low, they will not be discussed at this time.

OWN INVESTIGATIONS

Methods: In accordance with experience gathered earlier (Schäperclaus, 1970) the following antigen vaccines were prepared:

Endotoxins: Suspensions of highly virulent agar cultures were carefully killed with phenol or chloramphenicol. As a rule 1 ml contained 3 cm² of agar growth, corresponding to 1.5 x 10¹⁰ cells.

Exotoxoids: To peptone water cultures, kept under conditions assuring the greatest production of exotoxins, we added 0.4% of commercially available formalin. Subsequently the cultures were allowed to mature for three weeks at 37°C. Toxin formation was
constantly monitored by determining the minimal hemolytic unit (MHU). Using nutrient broth did not seem to offer any advantage as toxin formation does not exceed that obtained in peptone water. The bacteria grown in the nutrient solution were not filtered out; therefore, 'exotoxoid vaccines' actually also contained about $2.5 \times 10^8$ cells with endotoxins per milliliter. But, it cannot be assumed that 'endotoxic antigen' vaccines contained noticeable amounts of exotoxins.

The following experiments in carp were carried out with these two antigen vaccines or mixtures of both at a 1:1 ratio (containing $7.6 \times 10^9$ cells):

1. Aquarium tests with intraperitoneal or oral administration.
2. Experiments in eight 0.25 ha* test ponds with intraperitoneal injection when two-summer carp ($K_2$) was introduced in the spring.
3. Experiments in industrial warm-water production tanks with oral application in one-summer carp ($K_1$).

During 1970/71 six series of tests were carried out, each with 6 - 7 test aquaria (test groups) of 300 liters and a total of 600 carps. The test ponds were populated in 1970 and 1971 with 900 $K_2$/ha fed with 2300 kg rye grain/ha. At the time they were introduced each carp received 1 ml of vaccine i.p. Serological tests were done at that time and at the time of fish removal middle of October. Experiments in warm-water tanks containing at least 6 m$^3$ of water, were carried out in three different industrial type production installations each with 5200 - 6800 $K_1$ per tank. The water flow had a temperature ranging from 21 - 24 - 27°C. The oral vaccine, a mixture of exotoxoids and endotoxins, was sprayed on the feed

*ha = hectare; 1 ha = 2.471 acres
and distributed thoroughly. Vaccine was fed on three days each week over a six-week period: 0.4 liters/day per tank in the first week increasing up to 2-1/2 liters in the 6th week. On the average each carp received a total of 5 ml of vaccine, i.e. apart from the exotoxoids about 3.8 x 10^{10} cells. All technical work was performed by Technical Assistant Mrs. Brauer. In the practical industrial tests we had the support of the VE* Freshwater Fisheries Peitz and Kreba.

To determine the efficacy of each experimental vaccination, antigenic and immunogenic properties of vaccines were assessed by comparison with the condition of unvaccinated control carp of the same type. The antigenicity of the corpuscular antigen in suspensoid dispersion was monitored by determining humoral agglutininins. Precipitation furnished the yardstick for the antigenicity of colloidal-disperse, 'dissolved' antigens (formation of humoral precipitins). Development of immunity was studied in aquarium tests by the size of piece losses incurred in 'active protection tests' following intraperitoneal injection of a lethal dose of live homologous bacteria (whenever possible LD_{50} was determined). In ponds and warm-water tanks only the number of fish lost, multiplication and feed utilization could serve as 'measuring rod'.

The agglutination method described earlier (Schäperclaus, 1970) was used again. Precipitation: according to Ouchterlony's method in 1.5% agar with 0.7% NaCl. Since this method does not permit accurate quantitative determination of precipitins, the percentage of positive values was calculated as an indicator of the effectiveness of vaccination. Numerous duplicate determinations

* VE(B) = 'volkseigener Betrieb' (an industrial concern owned by the people), or in other words a state-controlled enterprise. A term used in Communist countries. (Translator).
did not reveal any differences in regard to agglutination or precipitation.

Particular attention had to be paid to the immunological status of test carp: the initial immunological condition of the fish at the start of an experiment plays an important role. Ideally only carp which are neither visibly affected by ascites at the time of the experiment nor have overcome it in the past should be used, but it is hardly possible to find such carp. To grow a special breed of 'sterile' carp would have been too expensive. Nor was it possible to determine the type and amount of all antibodies at the start of each experiment because the numerous and highly diverse serotypes of *A. punctata* are still not sufficiently well known. All we could do was to use the healthiest possible carp, and determine - at the outset - the agglutination titer for the antigens used as vaccines. As for precipitins, with their much lower specificity due to widespread antigen propinquity, only the incidence of positive precipitations was determined and given in percent. In other words, we were only able to record a rise in antibodies against the antigens used for vaccination.

Apart from ascites infection and its sequelae, other infectious diseases increasingly complicated the experiments. In the last aquarium test series, for example, 62% of Kₐ carp was lost during a four week accommodation period due to the disease referred to as 'gill necrosis', presumably attributable to myxobacterial gill infection (Fig. 2). Especially in prophylactic tests, the general health of the fish must be monitored carefully.
Fig. 1: Agar gel precipitation according to the method of Ouchterlony in test carp - Vetschau, October 5, 1970.
The central hole punched out of the agar contains the serum of the test fish and the precipitins. The two holes at the bottom are filled with abacterial peptone water cultures of Ez 7. They contain the exotoxic antigen of the Aeromonas punctata strain Ez 7. The two upper holes were filled with peptone water for control purposes. Two single white precipitation lines are visible between the central hole and the two holes at the bottom; the test is positive.

Fig. 2: Gill smear from test carps in the 6th aquarium test series in September 1971. The fish had been afflicted by chronic gill disease and necrosis since June. Staining with methylene blue. (The ubiquitous, massive thread-forming bacteria largely resemble pictures published on myxobacterial infections).
RESULTS

AQUARIUM TESTS

Table I illustrates the effect of adding Freund's adjuvant or aluminum hydroxide solution to the vaccine in a new test series. An intraperitoneal dose of 1 ml of the monovalent antigen vaccine Ez 7 was injected on December 6, 1969. Thirtyseven days later, on January 13, 1970, the carp weighing 180 g a piece were given the homologous lethal dose by intraperitoneal injection. Freund's adjuvant had enhanced the protective potency of the vaccine considerably: upon dissection of vaccinated carp most of it was recovered unchanged from the site of infection in the abdominal cavity. It was apparently attributable to this depot effect that the agglutination titer was lower than it was with vaccine to which no adjuvant had been added.

**TABLE I**: The effect achieved by adding adjuvant to the vaccine

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Carp surviving in active protection test on Jan. 18, 1970</th>
<th>Agglutination titer on Jan. 12, 1970</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>Combined vaccine + 50% Freund's adjuvant</td>
<td>89</td>
<td>220</td>
</tr>
<tr>
<td>Combined vaccine + 20% Al(OH)_3 (2.5%)</td>
<td>45</td>
<td>460</td>
</tr>
<tr>
<td>Combined vaccine without any additive</td>
<td>45</td>
<td>820</td>
</tr>
</tbody>
</table>

Tables III and IV reflect the degree of antigenic activity of various vaccines following parenteral or oral administration. Most test populations, 11 of 19, were fed 'exotoxoids' (which did, however, also include some endotoxins in the suspension). The other experiments where combined vaccines or only endotoxins were
fed, or vaccines were administered parenterally, and the controls primarily served for comparison.

In three of five cases the agglutination titers of control carp are strikingly high. As a rule this would be attributable to the fact that they have overcome ascites in the past. It should be borne in mind, however, that carp may also 'eat themselves immune'. It has long been known that fish meal may contain _A. punctata_; thus pellets may also comprize bacteria and their exotoxoids, and may act as antigens (see Table II).

**TABLE II: Effects of six week feeding of pellets**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Surviving carp in active protection</th>
<th>Agglutination titer against test with strain 1/53</th>
<th>strain Ez 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>44</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Feeding eel pellets</td>
<td>14</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>Feeding carp pellets</td>
<td>50</td>
<td>870</td>
<td></td>
</tr>
</tbody>
</table>

We were able to prove this hypothesis in the 5th and 6th test series which were maintained on beef and wheat grain, with pellets only used for comparison. Carp fed carp pellets clearly acquired immunity against _A. punctata_. Water left standing for 2 hrs. at 20°C with trout pellets yielded a positive reaction in the precipitation test with carp immune serum. Thus, pellets did indeed contain _A. punctata_ exotoxoids.

It is evident from Table III that intraperitoneal injection of 868 vaccines almost always produces a substantial rise in the agglutination titer. In a single exceptional case exotoxoids with very little suspended endotoxins were injected. Following oral administration of vaccines only a qualified rise in the agglutination titer can be detected. Of 11 test groups fed exotoxoids only six, i.e., 55% presented a relatively slight rise in titer. But, Table IV does show
### Table III: Antigenicity of vaccines. Mean agglutination titers in individual aquaria (test groups) on termination of immunization tests.

<table>
<thead>
<tr>
<th>Aquarienversuchsreihe</th>
<th>Zeit</th>
<th>Kontrolle</th>
<th>Intraperitoneale Applikation</th>
<th>Orale Applikation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>kombinierte Impfstoff</td>
<td>Endotoxin</td>
</tr>
<tr>
<td>I</td>
<td>Dezember 1969 bis Februar 1970</td>
<td>3000</td>
<td>0</td>
<td>820</td>
</tr>
<tr>
<td>III</td>
<td>Juni bis Juli 1970</td>
<td>3320</td>
<td>2000</td>
<td>3730</td>
</tr>
<tr>
<td>IV</td>
<td>September bis Dezember 1970</td>
<td>1200</td>
<td>3300</td>
<td>-</td>
</tr>
<tr>
<td>V</td>
<td>Dezember 1970 bis März 1971</td>
<td>1300</td>
<td>1400</td>
<td>1300</td>
</tr>
<tr>
<td>VI</td>
<td>April bis August 1971</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
</tbody>
</table>

1 = aquarium test series  
2 = time; 3 = controls  
4 = Intraperitoneal application  
   a = combined vaccine;  
   b = endotoxin; c = exotoxoid.  
5 = oral application  
   a, b, c as above.

### Table IV: Antigenicity of vaccines. Percentage of positive precipitations in various aquaria (test groups) on termination of immunization tests.

<table>
<thead>
<tr>
<th>Aquarienversuchsreihe</th>
<th>Zeit</th>
<th>Kontrolle</th>
<th>Intraperitoneale Applikation</th>
<th>Orale Applikation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>kombinierte Impfstoff</td>
<td>Endotoxin</td>
</tr>
<tr>
<td>I</td>
<td>Dezember 1969 bis Februar 1970</td>
<td>3000</td>
<td>0</td>
<td>300</td>
</tr>
<tr>
<td>III</td>
<td>Juni bis Juli 1970</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>IV</td>
<td>September bis Dezember 1970</td>
<td>30</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>Dezember 1970 bis März 1971</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>VI</td>
<td>April bis August 1971</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Dezember = December;  
Februar = February  
bis = to  
September  
März = March
that - compared to controls - precipitation is enhanced by oral vaccination. Of course, where 100% precipitation was recorded equally in control fish, it is impossible to judge the effect. Parenteral injection of combined exotoxoid-containing vaccines also yielded 100% precipitation.

Table V has been compiled to reveal whether the vaccines used are endowed with immunogenic properties and are capable of further enhancing pre-existing immunity in the carp. The active protection test is thus designed to demonstrate that parenterally or orally vaccinated carp is more resistant than unvaccinated carp. As a rule a homologous bacterial strain was injected in active protection tests but in some instances we investigated to what extent immunity against one strain also held up against another strain.

Table V shows that the number of all vaccinated carp lost in the protection test was lower than that of control fish. The percentage of surviving vaccinated carp - after the percentage of unvaccinated carp has been deducted - is always positive. Frequently somewhat greater immunity is acquired by intraperitoneal vaccination than by oral vaccination. Feeding exotoxoids consistently resulted in marked immunity and reduced the number of carp lost. Feeding endotoxins is not quite as effective; results obtained with combined vaccines did not show any significant differences.

In conclusion we are able to state that 'needleless' immunization of carp against *Aeromonas punctata* by feeding vaccines is feasible. In analogy with parenteral immunization, partial immunity the fish have already acquired naturally can be enhanced and further expanded, i.e. it can be extended to other types of *A. punctata*. Yet, immunity acquired by active immunization is never absolute.
### TABLE V: Immunogenic properties of vaccines.

**Efficiency of vaccination in active protection tests.**

Percentage of surviving vaccinated carp in various test groups after percentage of loss in unvaccinated control carp has been deducted.

<table>
<thead>
<tr>
<th>Aquaria</th>
<th>Zeit</th>
<th>Intraperitoneal Application</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>December 1969</td>
<td>Combined vaccine</td>
<td>9</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>February 1970</td>
<td>Endotoxin</td>
<td>30</td>
<td>b</td>
</tr>
<tr>
<td>II</td>
<td>February/May 1970</td>
<td>Exotoxoid</td>
<td>17</td>
<td>c</td>
</tr>
<tr>
<td>III</td>
<td>June/July 1970</td>
<td>Combined vaccine</td>
<td>18</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>September/December 1970</td>
<td>Endotoxin</td>
<td>8</td>
<td>b</td>
</tr>
<tr>
<td>IV</td>
<td>April/August 1971</td>
<td>Exotoxoid</td>
<td>16</td>
<td>c</td>
</tr>
</tbody>
</table>

1 = aquarium test series; 2 = time; 3 = intraperitoneal inj.  
4 = oral application; a, b, c same as under 3.

### TABLE VI: Studies on 5 labeled K2 from each of four test groups in experimental ponds in Kauppa on October 13, 1970.

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Agglutination titer against strain Ez 7</th>
<th>Precipitation with 8 vaccine antigens mean values</th>
<th>Percentage of positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exotoxoids</td>
<td>1060</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Endotoxins</td>
<td>1702</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Combined vaccine</td>
<td>1050</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Controls</td>
<td>350</td>
<td>27</td>
<td>27</td>
</tr>
</tbody>
</table>
It can be shattered by massive bacterial invasion. To what extent merely 'relative' immunity is adequate to prevent disease outbreaks under our conditions of production had to be determined by practical tests in some large-scale enterprises.

**Pond experiments in Kauppa**

The vaccines used in 1970 contained antigens of 8 different *A. punctata* strains. The agglutination titers always clearly reflected their antigenicity (Table VI) whereas precipitation did not reveal any differences by comparison with controls. Immunogenic properties of exotoxoids and endotoxins are only in evidence to a limited extent, especially as the population was apparently not hit by any severe disease.

When the carp were installed in 1971 they had a mean agglutination titer of 1:285 against strain Ez 7. At the time the ponds were emptied, the titer in control carp was 1:200, i.e. almost unchanged. Vaccine with Ez 7 + 1/53 yielded a titer of 1:1200 and vaccine with 5/47/50 gamma a titer of 1:3400. Thus the antigenicity of vaccines in 1970 and 1971 was very high even though by the time the ponds were emptied of fish, six months had passed since the intraperitoneal injection had been given. Precipitation was already largely positive at the time *K₂* were introduced. Apparently carp in the pond early form precipitins whereas *A. punctata* exotoxins are either produced in the intestine or contained in the feed. Nevertheless, minor differences were noted on October 26, 1971. Below we give the ratio of negative to positive precipitations:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>2.58:1</td>
</tr>
<tr>
<td>Combined vaccine Ez 7 + 1/53</td>
<td>0.8 :1</td>
</tr>
<tr>
<td>Combined vaccine 1/53 only</td>
<td>1.4 :1</td>
</tr>
<tr>
<td>Combined vaccine Ez 7 + 5/47/50 gamma</td>
<td>1.6 :1</td>
</tr>
</tbody>
</table>

It was difficult to evaluate the immunogenic properties of
vaccines, i.e. their actual potency, because the number of fish lost was apparently not so much attributable to ascites but rather to 'gill necrosis', air-bladder inflammations and other diseases (Table VII).

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Piece loss %</th>
<th>Total accretion kg/ha</th>
<th>Absolute feed quotient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exotoxoids</td>
<td>2.8</td>
<td>1694</td>
<td>3.0</td>
</tr>
<tr>
<td>Endotoxins</td>
<td>6.0</td>
<td>1638</td>
<td>3.2</td>
</tr>
<tr>
<td>Combined vaccine</td>
<td>10.8</td>
<td>1464</td>
<td>4.1</td>
</tr>
<tr>
<td>Controls</td>
<td>10.0</td>
<td>1464</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Experiments in warm-water tanks in Vetschau May to October 1970

No loss was recorded in the carp introduced at an average weight of 67 g and removed at a weight of 320 g; consequently no difference between vaccinated and unvaccinated fish can be observed. To determine the antigenicity of the vaccine, serological tests were performed on 10 and 12 carps respectively September 30 and November 4 (Table VIII).

<table>
<thead>
<tr>
<th>Type of vaccination</th>
<th>Agglutination titer against strain Ez 7 on Sept. 9, 1970</th>
<th>Percentage of positive precipitations with 8 strains on Sept. 30, 1970</th>
<th>Nov. 4, 1970</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1:160</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Oral vaccination</td>
<td>1:180</td>
<td>69</td>
<td>53</td>
</tr>
</tbody>
</table>

Experiments in warm-water tanks in Hirschfelde June to August 1971

The vaccine was composed of 1 part strain 5/47/50 gamma plus 1.4 parts each of strain 1/53 and strain Ez 7. On June 16, 1971 at the start of the experiment, 10 carps were examined. Mean agglutination against Ez = 1:66. Precipitations against the three
vaccine antigens were positive to 67%. Unfortunately one week after the experiments had begun, at a time when the fish had as yet been fed very little vaccine, the population was affected by a violent outbreak of ascites in conjunction with air-bladder inflammation. Loss in the entire installation which had only amounted to 100 pieces a day, rapidly rose to 700 and on July 5 already to 1700. On July 23 loss declined again to 1000 a day and by July 31, it had declined to 100. Throughout the entire period of the disease the carp fed satisfactorily; consequently, vaccine feeding was not interrupted before July 31. On completion of the test, piece loss in the three tanks of vaccinated fish was of the same magnitude as in the three tanks of unvaccinated fish. The loss amounted to 17.7%. Due to acute ascites, agglutination titers in control carp had tripled by August 17, 1971 (Table IX). In the vaccinated carp it had even increased 4.5-fold: oral vaccination had obviously been very effective. Bacterial strain 5/47/50 gamma though did not exhibit any antigenicity, and strain 1/53 a lower antigenicity than str.\textsuperscript{4} Ez 7. In tests with the three vaccine antigens, it was found that precipitation in the total number of all tests was 57% positive in control carp, and 46% in the fish fed vaccine. Thus, despite the ascites outbreak, the incidence of positive precipitation had declined somewhat.

**TABLE IX:** Mean agglutination titer in Hirschfelde August 17, 1971

<table>
<thead>
<tr>
<th>Type of vacc.</th>
<th>Antigens (bacterial strains)</th>
<th>Ez 7</th>
<th>1/53</th>
<th>5/47/50 gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td>183</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td>Oral vaccination</td>
<td></td>
<td>293</td>
<td>175</td>
<td>0</td>
</tr>
</tbody>
</table>
Experiments in warm-water tanks in Vetschau

October 1971 - 1972

In this experiment, it was possible for the first time to meet the requirements for unequivocal testing of oral vaccine activity. First of all, it was possible to select a uniform, relatively healthy K₁ population free of ascites, installed simultaneously in six tanks. Each of these now 8 x 1.25 m² large tanks, holding 10 m³ of water was populated with 14,600 pieces of K₁ (300 kg). Furthermore, vaccine feeding could be started as early as two days after the fish had been settled, and was continued for six weeks - 3 days each week. With an initial mean mass of 24-26 g per piece, the arithmetic mean of the vaccine dose received by each K₁ from October 21 to November 24, 1971 was 1.3 ml. Finally, all K₁ remained in the six tanks until December 11, 1971, i.e. for a total of 52 days, including 17 days following termination of vaccine feeding. Therefore, no chemotherapeutic agents were fed or injected. Subsequently fish in all tanks suddenly developed acute ascites accompanied by tumors.

This course of events permitted an unobjectionable evaluation on December 12, 1971. Even upon visual inspection, the disease in the four control tanks appeared to be about 3 times as severe as in the two tanks with fish fed vaccine. Careful inspection of 1000 fishes revealed that 3.5% of unvaccinated K₁ presented open tumors but only 1.25% of vaccinated carp. Of course, it was impossible to determine whether the 1.25% diseases vaccinated carp had not eaten enough vaccine or had been inadequately immunized.

It has thus been determined that while oral vaccination of carp does not provide absolute protection, relative immunity is acquired. The spread of the disease affected only about one third
the number recorded in unvaccinated fish. The same was borne out by the number of fish lost from December 12 to December 31, 1971, which amounted to a mean of 1.63 pieces per day and tank of vaccinated fish and 4.80 pieces per day and tank for unvaccinated carp. Weight increase for the period October 29 to December 28, 1971 was calculated at 113\% for vaccinated carp (initial weight 24 g) and 128\% (initial weight 25.3 g) for unvaccinated carp. The somewhat higher value in the latter case may conceivably be attributable to higher piece loss at equal amounts of feed and the slightly higher initial weight per piece.

Unfortunately, for economic reasons, it was not possible to pursue the full effect of the disease further in the 29,200 vaccinated and 58,400 unvaccinated carps. Chloramphenicol was added to the feed from December 15 to 21, 1971 to combat the disease: 50 mg/kg fish on the first day and 30 mg/kg fish on subsequent days. The disease declined at once and was almost suppressed completely by January 13, 1972. All tumors had healed forming large, black pigmented scars. On January 13, 1972 such scars were observed in 4\% of the vaccinated and 7.3\% of the unvaccinated fish.

The vaccine fed was a mixture composed to equal parts of the three antigens Ez 7, 1/53 and 5/47/50 gamma. Despite the acute ascites in all tanks, serological test results (Table X) show a considerable rise in agglutination titer in vaccinated carp. Striking are the wider margins of fluctuation which may be attributable to one carp getting more and another less or no vaccine. The only qualitative precipitations again point to a wide distribution of exotoxic A. punctata antigens, presumably in the feed. This probably also accounts for the fact that the differences between vaccinated and unvaccinated carp are not as great as in
Table X: Serological findings at the start and on completion of large scale warm-water tank experiments in Vetschau - Oct. 1971 - 1972.

<table>
<thead>
<tr>
<th>Art und Zeitpunkt des Testes</th>
<th>Mit Gemisch der 3 Antigene oral geimpfte Karpfen</th>
<th>Nicht geimpfte Karpfen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agglutinationstiter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>am 10. 10. 1971, Mittel von 25 K₁ (25 g)</td>
<td>925 800 1000</td>
<td>170 0 0</td>
</tr>
<tr>
<td>am 13. 1. 1971, Mittel von 4 K₂ (75 g)</td>
<td></td>
<td>175 25 630</td>
</tr>
<tr>
<td>Prozentsatz positiver Prazipiationen</td>
<td></td>
<td>66 100 100</td>
</tr>
<tr>
<td>am 10. 10. 1971, bei 25 K₁ (25 g)</td>
<td></td>
<td>91 73 82</td>
</tr>
<tr>
<td>am 13. 1. 1972, bei 8, bzw. 11 K₁ (75 g)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Art und Zeitpunkt des Testes = type and date of test
Mit Gemisch der 3 Antigene oral geimpfte Karpfen = carp orally vaccinated with a mixture of three antigens
Nicht geimpfte Karpfen = unvaccinated carp
Prozentsatz positiver Prazipiationen = percentage of positive precipitations
am = on; Mittel von = mean of; bei = at (in); bzw. = respectively.
CONCLUSIONS

Experiments in aquaria as well as large-scale tests in ponds and warm-water tanks have shown that carp can be immunized against *Aeromonas punctata* not only by parenteral but also by oral administration of vaccine mixed with the feed. These findings were borne out unequivocally by antibody formation against endotoxic antigens in suspensoid dispersion as well as exotoxic antigens in colloidal dispersion, and in active protection tests.

Obviously these problems are far too complex to be answered conclusively in studies like this. But, we have established that the use of combined vaccines containing *A. punctata* exotoxoids is also most important in 'needleless' oral immunization of carp. The results of these experiments suggest that in feeding experiments exotoxic antigens provide more immunity than endotoxic antigens. Moreover, they are less specific, and one can anticipate a wider spectrum of activity against numerous *A. punctata* serotypes or partial antigens. Overall the effectiveness of oral immunization is only slightly inferior to parenteral injection.

In analogy with conditions obtaining in Enterobacteriaceae and other bacterial species, immunity acquired by oral or parenteral vaccination is not absolute, only relative. It cannot be compared, for example, with the excellent efficacy of many antibiotics. It is beyond doubt, however, that in carp which have already naturally acquired more or less extensive partial immunity against a greater or lesser number of *A. punctata* types and partial antigen, immunity can be further enhanced by feeding vaccines.

To what extent such prophylactic vaccination can be effective in practice will always depend on the immunological condition of
the fish, i.e. whether they have already acquired immunity and against which types. Furthermore, the question arises how far they will be exposed to the risk of infection with new types during their continued growth. Needless, oral immunization is most promising in industrial warm-water tank fish production under the following circumstances:

1. If vaccine is fed early to carp or other fish spawns which have not yet had an opportunity to acquire adequate natural immunity against *A. punctata*.

2. Where new, highly virulent types of *A. punctata* appear in warm-water installations.

3. To enhance naturally acquired partial immunity further where the type of maintenance exposes carp to great stress and to a particularly high risk of bacterial infection.

**SUMMARY**

Experiments in aquaria, ponds and warm-water production tanks showed that it is possible to immunize carp against *Aeromonas punctata* by the parenteral or oral application of vaccine. In addition to endotoxic antigen, the exolaxoid content of a vaccine is particularly important. Immunization resulted in a considerable increase in the concentration of naturally-acquired antibody, and this extended its range of usefulness. The efficacy of immunization largely depended on the immune status of the fish and the conditions under which they were kept.
REFERENCES


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For translation of titles, see next page.
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9. Author's address.