DISEASE CONTROL AND REPRODUCTION OF GRASS CARP IN GERMANY

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INTRODUCTION

Grass carp were first imported into the Federal Republic of Germany from Hungary in 1964 - it was only a "good handful" of fry. The Wielenbach research station received the second load of 3-year old herbivorous fishes in 1966 (Bohl 1967). In the following years more fish were imported; not only from Hungary but from other countries. The many risks that are involved in introducing foreign species are now well known. There are not only ecological problems but also great dangers from disease. In contrast to the known disease problems, which are connected to the trade of indigenous fishes, the importation of foreign species often causes more trouble. These latter problems will be discussed.

NEW DISEASES IMPORTED WITH GRASS CARP

Not only new infectious diseases but also new parasites can be introduced by the import of fish, not native to our country. Most fish epidemics are not confined to one species. Therefore, the spread of fish epidemics caused by the import of non native fish is as great as those caused during the importation or trade of indigenous fish. Much more dangerous is the introduction of new parasites by the imported foreign fishes. However, it is only those parasites able to survive in a new biotop which will find conditions suitable for developing or where intermediate hosts occur. Fortunately, this rarely happens. Only during the first years after the introduction of grass carp into Russia were some species of parasites such as Crytobia branchialis, Capillaria amurasis found (Bauer et al. 1969). To these parasites the metacercaria of Metagonimus Yokogawai can be added. This metacercaria develops and becomes mature in the intestine of humans and of carnivorous mammals. Obviously the necessary intermediate hosts, certain molluscs, were missing in their new surroundings in Europe. Normally, only parasites become indigenous which do not need intermediate hosts or which are able to develop in several species or in a wide spread species. The introduction of new parasites to another country would not be so disturbing if these parasites remained with the imported species, but very often they are able to develop or live on other species of fish. In this respect, the recently invaded fish may be more severely damaged because they did not have occasion to develop immunity. The following helminth parasites have been introduced into the German Federal Republic by imported grass carp:

1. Bothriocephalus gowkongensis Yeh, 1955; the name of this species has been corrected by Körting (1975b) to B. acheilognathi Yamaguti, 1934.

in two week intervals under German carp pond conditions.

Another application-method used in Iran (Mokhayer 1976a) is 1 mg Yomesan per 1 g body weight of fish is cannulated into the first portion of the digestive tract. In a similar manner dibutyl-tindilaurate has been tried in New Zealand (Edwards and Hine 1974). If fish will not ingest food, this method is very helpful in cases of heavy tapeworm infestation.

Prophylaxe

Prophylactic measures involve controlling the Bothriocephalosis by disconnecting the life cycle. In this case eggs must be killed either by draining or disinfection of ponds. Kulow (1973) reported that the eggs of Bothriocephalus die very quickly in drained ponds at a temperature under 18 C. Disinfecting ponds thoroughly with quicklime (2.5 - 4 tons/ha) or chloride of lime (600 kg/ha) in combination with prophylactic measures are successful in ridding a pond of Bothriocephalus.

KHAWIOSIS

As already mentioned, the second of the two imported parasites was also a cestode. It is a caryophyllaeid. In routine examination it is very difficult to differentiate this new parasite, Khawia sinensis (Hsü 1935) from our European species Caryophyllaeus fimbriceps or laticeps. For identification the parasite should be fixed in 70% alcohol and stained with alauncarmine (50 mg ammonia potassium and 1 mg carmine heated in 1 L of water for 15 minutes and filtered). Before staining the parasite must be rinsed thoroughly in aqua dest. The preparation is stained for 5 - 30 minutes and rinsed again.

Microscopically the two parasites can be differentiated: The vesicles of the testes and vitellaria begin about 2-4 mm behind the scolex of Khawia sinensis, whereas the pre- and postovarian vitellaria occur in the cortical parenchyma in Caryophyllaeus, some postovarian vitelline follicles occur in the medullary parenchyma in Khawia sinensis. This can easily be seen in cross section. Obviously the difficulty in identification is one of the reasons that in the GFR Khawia has been found in only a few fish populations. Up to 60 parasites have been found in one fish. When fish are heavily attacked a catarrhalic or catarrhalic-hemorrhagic enteritis results. If secondary infections follow there are usually high losses. The life cycle requires oligochaeta (Tubificidea) as intermediate hosts and invasion is highest in early summer. The procercoid remains in the tubificid and is able to invade fish for about one year. During late summer (August) the cestodes leave the intestine and die.

The primary final hosts for this parasite are one year old carp and grass carp. Khawiosis is controlled like Bothriocephalosis[1/].

1/ After the Grass carp conference the following report was published: "Successful treatment of carp caviosis by means of Taenifugin in Czechoslowakia by Tesarcik, J. and A. Prouza, 1977. Bul VURH Vodnany 8(4):26-31. Taenifugin granulat: piperazin-niclosamide, sodium chloride and wheat-food-flour.

2. Khawia senensis Hsü 1935 (Körting 1974).

BOTHRIOCEPHALOSIS

The most dangerous species is the pseudophyllidian tapeworm, Bothriocephalus acheilognathi. While it damages the grass carp very little, it can develop in young European mirror carp. In some cases almost 100% of the one-year old carp are attacked.

In case of a heavy infestation, growth is reduced considerably. In the summertime mortality can reach about 30% and during the winter about 90% (Kulow 1973). Up to 60 tapeworms have been found in one fish. In Russia up to 3,000 in one fish. In this case the entire digestive tract is stuffed with tapeworms. This often results in a catarrhalic or catarrhalic-hemorrhagic enteritis. These young carp will appear small and very cachetic as compared to normal ones. In Germany, we have not found a heavy infection in grass carp, in contrast to the experiences in Iran (Mokhayer 1976b). The scolex of this tapeworm possesses two bothrias. The adult worm sheds eggs into the fish gut and into the water. The coracidium hatches at 25 C water temperature during the third day. Further development to the procercoid stage takes place in copepods (intermediate hosts) and is completed after 8-10 days (Körting 1975a). The life cycle of this tapeworm is completed when infected copepods are eaten by carp and other susceptible species. In the intestine it completes its development from procercoid to a plerocercoid-like stage after 20-21 days to the adult tapeworm of Bothriocephalus acheilognathi.

In Germany, this tapeworm has not been found in fishes other than carp and grass carp. In Russia, this parasite also lives in crucian carp (Carassius carassius), gibel (C. auratus gibelio), white bream (Abramis brama), barbel (Barbus barbus), chrup (Aspius aspius), wels (Silurus glanis), and species of gambusia (Bauer et al. 1969). Reasons for the rapid spread are: 1) a relatively simple life cycle; 2) several cyclops species as intermediate hosts; which are widely spread over the world; and 3) the wide range of final hosts.

To prevent damage in fish and further spread, it is necessary to control Bothriocephalosis.

CONTROL OF BOTHRIOCEPHALOSIS

Direct Therapy

There are several medications for direct therapy:

Phenothiazin (1 g/kg body wt per oz; 3 time ia intervals of 2 days).

Di-n-butyl-tinoxyd (0.5 g/kg body wt 1 to 3 times a day for 3 days (Hnath 1970).

The best medication is Mansonil^{R}, a niclosamide-piperazin-salt (a synonym is Yomesan^{R}, used in human medicine). This substance is mixed into the food; 500-600 g/100 kg dried pellets, 1.5% of body weight is fed 2 to 3 times

Cryptobiasis. Against the first two protozoans malachite green is successful (0.15 ppm; 1-3 times in intervals of two days). Sometimes in therapy malachite green is combined with 24 ppm formaldehyde. According to Russian experiments (Zobel 1975) treatments with brilliant green and violet K (arylmethan-colour substances) are also very effective in concentrations of 0.15-0.2 ppm (water temperatures 7-12 C) against Ichthyophthirius, Childonella and other parasites. Against the gillworm Masoten^{R} (Metrifonal, 0.5 ppm = 5 kg/hectare average water depth of 1 m) is very effective. Short term baths can also be given with sodium chloride (2.5%, 10 minutes) or better results are obtained with formaldehyde (20-25 ml of 40% formaldehyde in 100 litres of water for 30 minutes) (Bohl 1975).

We have also had losses of grass carp fingerlings due to cryptobia, a flagellate protozoa. During heavy infestations fish showed typical "Scale detrusion". On the sides of their bellies there were many small hemorrhagics and the skin was slimy. The epithelium of the mouth was swollen with many cryptobia present. In some cases they were found only on the gills.

PROPHYLAXE FOR INTRODUCED PARASITES

Imports of cyprinids, which are already on food, should be denied entry. Fry are excluded from this edict if they are hatched in closed warmwater systems and are fed on artificially produced food. The reason for this rule is that all species of fish could introduce several parasite species into the country. Older fish carry more parasites. In spite of intensive diagnostic controls of imported fish, we found that generally only 25% of the possible parasites are found.

During a period of quarantine of 2 to 4 years about 75% of the parasites are found. About 100% are found after 5 to 10 years. The most dangerous parasites, Bothriocephalus, Khawia and Philometra belong in these categories.

In spite of increasingly stronger control regulations and consequent quarantine, 27 different species of parasites were introduced into Russia (Bauer et al. 1969). Rinsing the intestine may help considerably when diagnosing intestinal parasites of living fish (Faina and Par 1977). A small plastic hose is introduced into the cranial part of the digestive tract and is connected to a syringe in order to rinse the intestine with water. Carp, with a weight of more than 20 grams can be examined by this method. It is more difficult with grass carp. Food as well as cestodes will be washed from the intestine. This may not be the best method for diagnosis, but in some cases it is helpful.

INFECTIOUS DISEASES

As previously mentioned, infectious fish diseases can be introduced and spread by foreign fish. It is known that grass carp can be affected by infectious dropsy, which may sometimes result in heavy losses.

The dropsy-complex is divided into two types: "spring viremia", the acute form - the infectious agent being Rhabdovirus carpio - and erythrodermatitis, the chronic form - the infectious agent being a subspecies of Aeromonas salmonicida var. achromogenes. A specific enteritis is also observed in the

OTHER PARASITOSIS

Because laws do not exist for the control of fish imports, fish epidemics are uncontrolled. If major losses do not occur, imported fish will be without health-control. Therefore, we may assume that other foreign parasites have been introduced into our country by the imports of grass carp or carp. In this manner, 27 species of parasites have been introduced into Russia (Bauer et al. 1969). Most of them are protozoans and are not usually as important[2/].

Another example of a dangerous parasite is a nematode: Philometra lusiana (Vismanis 1966). The red colored female penetrates through the skin where the posterior end can often be seen. The males usually enter the caudal part of the airbladder and destroy it. Fish infected in this manner lose their equilibrium.

DISEASES WHICH AFFECT GRASS CARP IN EUROPE

Not only are indigenous fishes endangered by imported disease agents or parasites, but imported fishes are endangered because they may not be immune against our indigenous parasites and other disease agents.

We know of some cases where grass carp have been heavily infested with digenetic trematodes. From Rumania (Fadulescu and Georgescu 1963) and the German Democratic Republic (Mattheis 1967) heavy invasions of metacercariae of Diplostomum spathaceum have occurred, which causes parasitic-cataract ("Wurmstar"). Grass carp are invaded 5 to 25 times as much as carp which are the same age and reared in the same ponds. In Hungary the situation is similar (Szakolczai and Molnar 1966) with about 200-300 parasites per host. Grass carp are more susceptible to these metacercaria than carp.

In the German Democratic Republic grass carp were strongly invaded by a tetracotylous metacercaria (Apharyngostrigea curnu) in the abdomen (Mattheis and Odening 1969). Along with other species of fish the grass carp is the second intermediate host for this parasite. Obviously the grass carp is preferred by the miracidia, for they develop very well. The first intermediate host is the common pondsnail (Planorbis sp.). The final hosts are heronbirds. A heavy infestation by a similar species of metacercaria (Tetracotyle percae fluviatilis) caused heavy losses in Hungary (Szakolczai and Molnar 1966).

These tetracotylosis are controlled by interrupting the life cycle. In this case the best method is to control the snails with molluscicides (Bayluscid^{R}, 0.5 ppm) or possibly with snail eating fish like Mylopharyngodon piceus, and by keeping away the final hosts (waterbirds). Other widespread parasitosis which endanger the population of the grass carp are: Ichthyophthiriasis (white spot), Chilodonellosis, Dactylogeirosis, and

[2/] Latest experiences have shown that Balantidium ctenopharyngodonis - belonging to the suborder Heterotrichaea - was found in the intestines of grass carp and common carp at our research station (Klein, 1978; pers. communication).

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grass carp.

To prevent secondary infections the following treatments with antibiotics are advisable: by feed, injections or baths (Amlacher 1970, Bohl 1970).

a) Medicated feed - Oxytertracyclin 3500-7500 mg/kg food 10 - 14 days (50-75 mg/kg body wt, 7 - 10 days). The lowest concentration which helps against Aeromonas punctata in the blood is 0.1 γ.

b) Injection - Chloramphenicol or Oxytetracyclin 20-25 mg/kg fish in 0.5-1 ml aqua dest. intraperitoneal. The depot-application of antibiotics 400-500 mg Chloramphenicol/kg body weight, mixed with 1 ml mixture of paraffin-oil (80%) and Ianolin (20%) intraperitoneal is the best protection against an outbreak of infectious diseases (Vladimirov 1969).

c) Bath - 100 mg/l for severe cases and normally 60-80 mg/l Chloramphenicol up to 36 hours with aeration. Sulfadiazin 100-250 mg/l water, long duration bath. Resistance tests should be taken to determine the most effective therapeutics.

In some cases branchiomycosis broke out. The fungus Branchiomyces sanguinis grew very rapidly; high mortalitys often occurred.

Therapy with quicklime (150-250 kg/ha) or copper sulfate (about 1 mg/l; toxicity depends on water chemistry) can be used.

Furthermore, a colleague in Munich isolated a rhabdovirus from grass carp collected in the River Danube. This rhabdovirus was previously unknown (Ahne 1975). This virus is highly infectious (up to 100% mortality) in grass carp fry, but not in older ones and not in carp. It differs in serological characteristics from RVC and shows a resemblance to pike fry virus (Ahne 1977, personal communication). It should also be mentioned that in the United States they have had trouble with columnaris disease (Shireman et al. 1976).

Russian experiences have shown that the RVC is most likely not transferred by eggs and sperm. For this reason, artificial breeding and rearing of fry can take place when hygenic methods are followed. Together with colleague Ahne, from Munich, we shall work on the different aspects of these problems this season.

REPRODUCTION OF GRASS CARP

At our research station we perform experiments on the biotechnical reproduction of grass carp in a warm water recirculation plant. This will enable us to obtain disease-free fry and become independent of imports. Since 1974, grass carp have been bred at Wielenbach. Until recently, our warmwater plant was small and our work was limited. Nevertheless, this year we will start a large warmwater plant. One cycle with activated sludge, two others with trickling filters.

Our present research plant uses a gravel filter system - it is a very simple system, but for our purposes it was sufficient.

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