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Praziquantel treatment of captive white-spotted eagle rays (*Aetobatus narinari*) infested with monogenean trematodes

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Abstract

Captive white-spotted eagle rays (*Aetobatus narinari*) were infested with *Clemaoityle australis* (Monogenea: Monocotylidae) on the skin and with a species of the Hexabothriinae on the gills. Different behavioural changes have been described caused by these infections. Oral treatment with 10-40 mg praziquantel per kg bodyweight had no effect. Bath treatment with 20 mg praziquantel per litre for 45-90 minutes showed good results against both parasite species. The presence of cleaner wrasse (*Labroides dimidiates*) in the display tank decreased the infections of the rays.

Introduction

Trematodes belonging to the Monogenea have been described from many elasmobranchs in captivity. A good overview is given by Cheung (1993). Infected fish may die if they remain untreated. Treatment with praziquantel has been proven to be effective against monogenean infections (Schmahl and Mehlhorn, 1985; Schmahl and Taraschewski, 1987; Schmahl et al., 1989).

White-spotted eagle rays (*Aetobatus narinari*) kept at Burgers' Zoo in Arnhem, the Netherlands showed behavioural changes and proved to be infected with monogeneans. The aim of the present study was to describe the different behaviour of the infected rays and to measure the effect of a praziquantel treatment. As an alternative method for chemotherapy, cleaner wrasses (*Labroides dimidiates*) were introduced in the display tank with the rays.

Materials and Methods

Origin and maintenance of the rays

Eight white-spotted eagle rays, originally from the Maldives, were kept at the quarantine facilities of Burgers' Ocean, Arnhem, the Netherlands. The rays were kept in three round black tanks (diameter ca. 3 m, water height ca. 1 m). Water temperature was between 25° and 26°C, pH between 7.9 and 8.1, salinity between 33 and 34 ppt and O₂-concentration between 95 and 98%. Body width of the rays was between 37 and 56 cm, their estimated body weight between 0.5 and 4.0 kg. After 2 to 4 months in quarantine the rays were transferred to a display tank of 1.5 million litre. Water temperature was between 25.0° and 25.5°C, pH between 7.9 and 8.1, salinity between 33.0 and 33.5 ppt and nitrate levels at 20-25 mg NO₃⁻-N/l.

Feeding of the rays

The first month after arrival, the rays were daily fed three times a day, 7 days a week, with mussel (*Mytilus edulis*), razor shell (*Ensis siliqua*), gamba (*Penaeus* sp.), squid (*Loligo* sp), Atlantic herring (*Clupea harengus*), whiting (*Merlangius merlangus*), mackerel (*Scomber scombrus*), scad (*Trachurus trachurus*) and ansjovis (*Engraulis encrasicolus*). In the second month of quarantine the rays were trained to feed individually from a stick. After 2 months, the rays were fed twice a day for six days per week with the same food items as in the first month. These items were alternatively offered to encourage a variable diet and to avoid food preference.

Treatment

Due to behavioural changes and the subsequent demonstration of monogeneans on the skin, it was decided to treat the rays with praziquantel.

Oral treatment

The first oral treatment was given at a dose rate of 10 mg praziquantel per kg body weight (kgBW). The drugs were offered as a part of a tablet in the food items (in the tail of the gambas or under the skin of food fish). Possible regurgitation was carefully observed. The effect of the treatment was measured on the basis of presence of monogeneans in skin scrapings of the dorsal side of the pectoral fins taken before and after treatment. Because of lack of results, the study was repeated with an oral dose of 40 mg per kgBW.

Bath treatment

Due to the lack of results with the oral treatment, bath treatment was given. We started with a dose of 20 mg praziquantel per litre

for 90 minutes (Noga, 2000; J. Coreira, pers. comm.). In the first trial, the bath (25°-26°C) was aerated with pure oxygen to be sure that the O₂-content stayed above 80%. The results were not very promising, therefore, to avoid the possible influence of the pure O₂, the trial was repeated with compressed air.

Treatment in the display tank

When fish are kept in a display tank it is difficult and stressful for the fish to take them out. Bath treatment is under those conditions no option and therefore it was investigated if the presence of cleaner wrasses (*Labroides dimidiatus*) would be of influence on the external appearance and behaviour of the rays.

Results and Discussion

Behavioural changes

Within a couple of days after arrival, some of the rays showed a stressful behaviour that became worse within two weeks. This was expressed by:

- tips of the pectoral fins were kept high above the median line during swimming;
- spinning movements where the body was held in a vertical position;
- tail and pelvic fins were angled upwards during swimming;
- jumping out of the water against the net coverage of the tank;
- swimming against the current close to the water outlets;
- upside down swimming in one extreme case;
- regular resting on the bottom;
- scratching with the ventral side on the bottom of the tank.

Praziquantel dose (mg/kg body weight)	Mode of application	No. treatments	No. consecutive days treatment	Effect
10.0	Tablet	4	1	None
14.3	Tablet	2	1	None
16.7	Tablet	2	1	None
20.0	Liquid	1	1	None
25.0	Tablet	2	2	None
40.0	Tablet	1	1	None

Table 1. Oral treatment with praziquantel of 7 captive white-spotted eagle rays (*Aetobatus narinari*) infected with monogenetic trematodes

This behaviour caused haemorrhages on the tips and sides of the pectoral fins and on the skin under the pectoral girdle. Successful treatment was given by oral dosing of enrofloxacin (Baytril®, Bayer) at 10mg per kgBW per day during 7 days. The haemorrhages healed within a month. However, the skin became paler in colour. Skin scrapings showed that the colour change was due to a thick layer of mucus. Large numbers of monogenetic trematodes identified as *Clemacotyle australis* were present. One ray died 58 days after arrival due to the monogenean infection and total exhaustion. This parasite has been earlier described by Beverley-Burton and Whittington (1995).

Treatment

Treatment of monogeneans in elasmobranchs is often done with trichlorphon (Gruber and Keyes, 1981; Stoskopf, 1993). However, we had bad experiences with the treatment of a cow nose ray (*Rhinoptera bonasus*) and a white-spotted eagle ray who died after bath treatment with Trichlofon Tugon®80 (Bayer) at a dose of 0.2 mg per litre in a mixed elasmobranch display tank. Necropsy showed in both animals large congestions in their gonads. Therefore we decided to try oral and bath treatments with praziquantel (Droncit®, Bayer).

Oral treatment

The results of the oral treatment are presented in Table 1. Skin scrapings after the treatment did not show a reduction in the parasite infestation. Possibly the treatment should have been done over a longer period to get better results.

Bath treatment

The results of the bath treatment are shown in Table 2. The treatment gave much better results than the oral treatment. The treatment accompanied with oxygenation with pure O₂ gave some result, but less than with aeration. The treatment resulted in a normal behaviour of the rays. After treatment in combination with aeration, many parasites could be collected from the bottom of the tank. Identification showed that not only *C. australis* was present, but also a species belonging to the subfamily Hexabothriidae. Because these parasites were not found in the skin scrapings, it is very probable that they parasitized the gills.

All parasites on the bottom of the treatment tank were still alive and remained so for at least four hours. The effect of treatment was that they lost contact with the host, maybe due to temporary paralysis of the muscles in-

Conc. (mg praziquantel/l)	Oxygenation /aeration	Treatment time	Results (decrease in parasite no.)		
			None	Some	Complete
20.0	100% O ₂	90	2	2	0
19.3-20.5	Air	90	0	1	3
19.4-20.5	Air	45	0	1	1
25.0	Air	45	0	0	2

Table 2. Bath treatment with praziquantel of captive white-spotted eagle rays (*Aetobatus narinari*) infected with monogenetic trematodes.

involved with their clamp mechanism. It is not clear if these "free living" parasites can clamp to their host again during a subsequent contact. Maybe that a higher dose of praziquantel would kill the parasites, but at the dose of 25 mg/l the rays became already disoriented and were breathing heavily, so that a higher dose may be toxic for the rays as well. The disorientation may also be caused a pH drop (approximately 0,3-0,5 pH decrease) of the water during the treatment.

Biological treatment in the display tank

After the bath treatment, the 7 rays were kept for 20 months without problems in a multiple species display tank. Gradually some rays developed pale greyish patches on the dorsal side, identical to the colour change observed in the quarantine period when infected by skintrematodes. The rays were caught from the display tank and a skin scrape was done. On six rays, juvenile *C. australis* could be found and on two rays also adult worms. On one ray no skinparasites were found.

Possibly the effect of the bath treatment was not 100% and living parasites were brought with the rays into the display tank. Also the display tank may have become infested by the trematodes due to the introduction of two infected rays one months after their arrival. It

was thought that part of the problem was caused by small confined space of the quarantine tank. This way two animals were released in the large display tank creating less confinement stress. Within one week they had to be removed from the tank since feeding became a problem.

Eight cleaner wrasses (*Labroides dimidiatus*) were introduced to reduce the trematode infection. It was observed that the cleaner wrasses regularly "cleaned" the skin of the rays and gradually the skin colour lost its pale greyish colour and became darker. Therefor indicate that the cleaner wrasses were able to control the parasites on the skin. Only one ray had to be removed from the display tank and was treated in a bath after which he was returned to the tank.

Oral treatments with praziquantel proved to be ineffective against skin monogeneans (*Clemaecotyle australis*) of white-spotted eagle rays (*Aetobatus narinari*). Bath treatment of 25 mg praziquantel for 45 minutes with aeration proved to remove the parasites from the skin, although the parasites did not die as they were found alive on the bottom of the tank. In big display tanks, cleaner wrasses (*Labroides dimidiatus*) seem to be able to control the parasite infection at a level which is not harmful for the rays.

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