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RESEARCH ARTICLE

DIAGNOSTIC IMAGING AND TREATMENTOF KOI ABDOMINAL INFLAMATION CAUSED BY DYSTOCIA

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ABSTRACT

Koi (*Cyprinus rubrofuscus*) is a domesticated colored variant group of Amur Carps originally found in Asia and Central Europe. The colorful scale pattern has made Koi fishes one of the most popular aquarium fishes worldwide. A case of a female Shusui Koi aging 7 years old was brought to Dr. Fish clinic located in Tehran, Iran. The owner has reported 3 months of abdominal inflammation, no problems with feeding or balance and no sign of sickness. In the clinical examinations and microscopic investigation via wet mount, skin and both gills were found healthy. Lateral and dorsoventral radiographs were taken. Both graphs showed a displacement of swim bladders due to the presence of tumor-like mass surrounding them. Sonography was done in order to further identify the mass. Swim bladder was found to be completely out of place due to the accumulation of eggs (egg binding) in the abdominal cavity. The egg bound Koi had been unable to release the eggs for months. The owner was advised to give the fish a short time to release the eggs naturally. When the natural course did not take place, the fish was brought back to the clinic for the first step of hormonal therapy. An intraperitoneal injection of GnRH (Ovaprim™) was done under the pectoral fin. The first injection dose contained 10% of the therapeutic amount. The next and final injection containing the remaining amount of the hormone, was done 24 hours later. The Koi was able to release the eggs shortly after the treatment.

INTRODUCTION

Nishikigoi fishes commonly known by the name of koi, are a colorful variety of Amur carp (*Cyprinus rubrofuscus)* that have been kept in water gardens or koi ponds for decorative purposes and were originally found in Asia and Central Europe. Koi varieties differ in color and scale pattern. The possible colors are virtually limitless, a fact that makes koi fishes one of the most popular aquarium fishes worldwide. New varieties are still being actively produced but breeders have identified and named a number of specific variations. One of these varieties is Shusui koi (meaning "autumn green") which was created in 1910 by Yoshigoro Akiyama. This fish does not have any scales, except for a single line of large mirror scales dorsally, extending from head to tail. The natural breeding of koi happens in the spring and summer seasons. The male will follow the female by swimming right behind the female koi and nudging her. After the release of eggs by female koi, the eggs sink to the bottom of the pond and stay there due to the existence of a sticky material surrounding the eggs. Female koi produces a vast number of eggs at a time, which can lead to dystocia (egg retention) if not released properly.

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What is Dystocia?

The terms "dystocia", "egg retention", "egg impaction" and "egg binding" all refer to the inability of a female to release the formed eggs through the reproductive tract. There are a number of reasons both external and internal that can cause dystocia, such as tumors, adiposity, and absence of oviduct,but there are two environmental reasons that are witnessed more frequently:

1. Similar to any other species, fish needs to be physically mature prior to the spawning. If the body does not grow into appropriate size before the process, it is possible that the eggs will be trapped inside of the body. Allowing the fish to mature also helps the ovaries to develop the ability to release eggs without them accumulating.
2. The temperature of the environment matters greatly. It needs to be 20o c as the ovulation starts and throughout the entire egg maturation process as well.
3. Fish are generally more sensitive towards changes in the pool they are kept in. Any change in the water quality or the location of the pools can cause distress, even if the change is not significantly effective on health matters.

Other than the reasons above, carp species have a unique characteristic that the release of the eggs requires the male fish to contact the female strongly. All together Koi is even more susceptible to dystocia than many species. If such problem occurs, it can only be diagnosed through different imaging techniques. Radiography is one of the non-invasive imaging techniques used for providing information on internal disorders in fish. Cases that are presented for radiography can include body shape anomaly, foreign bodies and buoyancy disorders. Equipment and techniques used for diagnostic imaging of fish are the same as domestic animals. "The use of rare-earth intensifying screens and high definition film is recommended due to the small size of many patients and tohelp identify subtle radiographic features" (William, 2003). While radiography is a useful technique to scan body for anomalies, it cannot help with identifying soft masses such as herniated organs, ovulation problems or any anomaly with the same brightness level as the rest of the body. To further identify the problem, an ultrasonogram might be needed. Ultrasonography is a safe method that provides a fine image of internal anatomy with the use of acoustic energy. Images are created via different methods of sonography:

1. **Amplitude Mode:** A-Modeproducesa one-dimensional graph of echo amplitudes,displayed as spikes on a vertical line. This method is best used in ophtalmic examinations and least used in reproduction related studies.
2. **Brightness Mode:** B-Mode produces a grayscale moving image that depending on the shape of the sector, can be shaped as a triangle or a rectangle. This method is commonly used for aquiring data of the internal organs of animals, including different types of fish,
3. **Motion Mode:** M-Mode is a modified B-mode that allows the researcher to evaluate moving structures such as heart, motion of the dots or a change in the distance between the probe and the studied interface.

MATERIALS AND METHODS

The patient was brought to the clinic carried in a plastic bag containing the original water of its tank and the bag was placed inside a Styrofoam box (Figure 2). In the clinical examinations and microscopic investigation via wet mount, skin and both gills were found healthy. After the standard evaluation of the patient, diagnostic imaging of the abdominal area was found to be needed for a better determination of the cause. Preparation of the devices and supplies was taken care of before the process of transferring the patient took place. To prevent the patient from moving and causing disturbance with the imaging process, extract of carnation flower (*Dianthus caryophyllus*) was added in 0.1ml portions until the fish showed signs of ataxia. Latex gloves with no talc were then used to carry fish to the vessel by which it would be transported. A movable cart was chosen for transporting patient and the supplies to the site of work.

The koi was then quickly removed from the vessel and placed on the plastic covered cassette, ready for the imaging process. The koi was positioned in right lateral recumbency to provide a clear view of the abdominal area. Although the anesthetic restraint method was used for the patient, anesthetic properties were present in the site in case of need. The fish was unconscious during the process, however, the lights in the radiography area were dimmed so as not to trigger a photophobic reaction. The patient was monitored throughout the radiographic exposure in order to prevent the fish from moving out of place. "Two views are recommended and at North Carolina State University; a standard left to right lateral and de- cubital VD/DV" (Love, 1997, p. 26). Whole body right lateral and dv radiographs were made to determine the extent of the swelling site (Figure 3). Diversion of the spine was observed due to the extra weight of the mass carried.

Figure 1. Body asymmetry and swelling of the abdominal area in Koi (a) Top view of the whole body with no signs of trauma or infected lesions (b) Front view of the fish, swimming without showing signs of distress

Ultrasonography together with radiography can provide wholesome infromation on internal organs and thus, these methods are commonly used in fish as well as the other animals. A case of a female Shusui koi aging 7 years old was brought to Dr. Fish cliniclocated in Tehran, Iran. The owner had reported 3 months of abdominal inflammation, no problems with feeding or balance and no sign of sickness. The abdominal area was observed as asymmetrical with an obvious inflamed area on the lateral side of the koi (Figure 1). The fish was kept at the clinic for further examinations and the course of treatment.

The graphs showed a total displacement of swim bladder due to the existence of the mass. No specific anomaly was observed in the ribcage or spinal cord.There was no evidence of any exogenous factors being present in the inflamed area. Further examinations via ultrasonography were needed to determine the cause of the abdominal swelling. Immediately after the radiography, the unconscious fish was carried to the ultrasonography site with the same cart that was used for the previous transportation.

Figure 2. Transporting the fish using original water of the tank in the bag

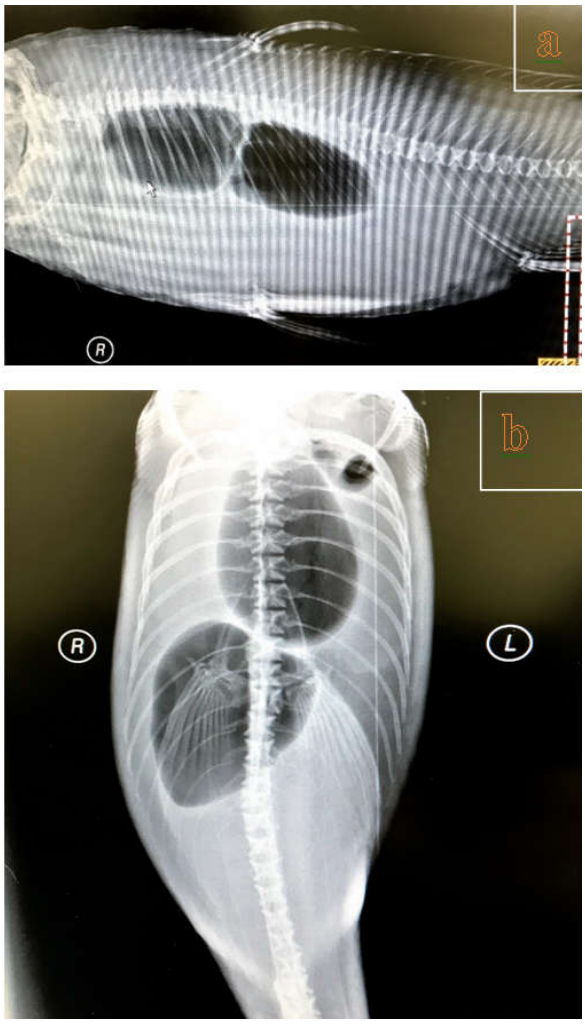

Figure 3. Radiograph of a Koi with an abdominal inflammation due to egg retention (a) Right lateral radiograph of the patient showing no evidence of external objects in the area (b) DV radiograph of the same patient further providing authors with information on the shape anomaly caused by egg retention in the fish

The fish appeared to be moving so it was placed once more in its tank that contained the anesthetic agent. It was then, carried inside the Styrofoam box to the site of imaging. To prevent the patient from early recovery, the fish was placed in lateral recumbency on the hand of the researcher inside the box, in a position that allowed it to have intake of anesthetic agent while allowing the process of imaging. Probe was inserted on the site of inflammation. Monitoring the site confirmed that there were no exogenous agents involved in the inflammation. Ovaries were found to be oversized and a clear view of the eggs confirmed a case of dystocia had occurred in fish.

RESULTS

Patient was given a week-long period of time to determine whether the eggs would be released without the administration of hormones. “Some females will spawn spontaneously after their return to the pond when they arekept isolated for a couple of days. The males will be stimulated strongly by this andhelp them to emit the eggs” (Lechleiter *et al.,* 2016). After the given time, the problem persisted and the patient was brought back to the clinic to receive hormonal therapy. Many of the captive fishes are unable to breed in captivity due to environmental differences with their natural habitat. Intervening is necessary for stimulating the ovulation in many of the cultured species. The owner of the fish mentioned the lack of interest in professional breeding of the species. The farm he owned did not separate female and male koi fish and it did not keep track of the population well. He also reported that the fishes were moved frequently from one pool to another. This can mean that the egg retention was caused due to the stress of moving in fish. Treatment was discussed then. A number of therapeutic agents exist for the treatment of egg binding. Drugs and dosages are listed in the Table 1. There are two common approaches for the treatment of dystocia in fish. Both GnRH and fish pituitary extract induce ovulation. Pituitary extract is prepared through removing the pituitary from a fish (such as carp, catfish, salmon) and extracting the hormones which will be injected into another fish. However, there are advantages to using GnRH:

a) GnRH is found in the market much easier than fish pituitary extract
b) The formulation is purified and more stable than pituitary extract
c) It is significantly less expensive than the alternative

Thus a treatment via GnRH (Ovaprim™) was considered. Ovaprim™ is a potent ovulating/spermiating agent used to promote and facilitate reproduction of many species of fish. It is a stable solution that contains GnRH and a dopamine inhibitor. Time to ovulation following Ovaprim injection is highly predictable, with high egg fertility and viability. Administration was done in two sessions of injection. An intraperitoneal injection of GnRH was done under the pectoral fin.The first injection dose contained 10% of the therapeutic amount. The next and final injection containing the remaining amount of the hormone, was done 24 hours later. After the administration of Ovaprim™, it is necessary to stimulate the release of eggs through abdominal palpation. The Koi was able to release the eggs shortly after the treatment. With hormonal injection administered, it was necessary to make sure that the dead eggs do not affect the water quality negatively. Water exchange every day may be needed. The female will need time

to recover. Vitamins and highly digestible low protein food may help the process of recovery. A second ultrasonography is suggested a week after the fish has released the eggs. This is to determine whether there are any mature eggs left in the abdominal area and whether there is a need for a repeated treatment process.

Table 1. Hormonal stimulation of egg release

Agent	Dosage	Comments
Pituitary preparations (1)	1 pituatry has about 2,5-3,5 mg/piece 1-10 mg are needed for 1 kg of fish repeat every 12 h inject i.m.	effective directly on the level of ovary may be combined with HCG (human chorionic gonadotropin) 20 IU/kg. Does not cause eggs to mature!
Pituitary extract (2)	5 mg/kg i.m. every 6 h	effective directly on the level of ovary may be combined with HCG (human chorionic gonadotropin) 20 IU/kg. Does not cause eggs to mature!
HCG (2)	30 IU/kg when used without Pituitary extract 20 IU/kg when used together with pituitary extract	Does not cause eggs to mature!
Haloperidol (2)	0,5 mg/kg i.m.	dopamine blocking agent used together with LRH-A to stimulate egg release.
LRH-A (2)	2 µg/kg i.m. after 6 h another 8 µg/kg i.m.	Does not cause eggs to mature Sometimes more effective together with haloperidol or reserpine
Reserpine (2)	50 mg/kg i.m.	dopamine blocking agent used together with LRH-A to stimulate egg release.
Metoclopramid (3)	20 mg/kg	neurolepticum and dopamin blocking agent used together with Buserelin (GnRH-A)
Buserelin (3)	10-20 **µg/kg** i.m.	GnRH-A (peptide) about 10x more efficient than GnRH

Conclusion

In the research done on the environment in which the fish was kept, no anomaly was observed other than the frequent moving of the fish, thus, it still remains unclear whether the cause of dystocia was distress alone or other factors such as genetic, a sudden change in water temperature and quality or else. Since the administration of hormones in fish with immature eggs can be a massive distress for the female and will fail to treat the problem, alternative methods are often suggested before the therapy such as letting the female release the eggs in a separate space from the rest of the fish and in the presence of a male Koi. If all the natural methods fail, the hormonal therapy is used. If this method fails as well, laparotomy and operation may help with both diagnosis and treatment. Dystocia may be witnessed regularly in the fish farming industry, however, lack of attention and leaving the problem unresolved may end in a bacterial infection that may enter blood circulation and cause a fatal septicemia, therefore, clinical examination in every case of abdominal shape anomaly is suggested.

In the matter of treatment, researchers continue to look for a safer method to stimulate ovulation in egg bound fish. Since the use of hormonal therapy is contradicted in the egg bound fish with immature eggs and the high stress levels that accompanies the treatment, the need to find a better solution still remains. Researchers hope to find more data and better ways of dealing with this particular problem in future.

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