한국어병학회지 제30권 제2호 (2017)
J. Fish Pathol., 30(2) : 97~105

www.ksfp.org
pISSN 1226-0819, eISSN 2233-5412
http://dx.doi.org/10.7847/jfp.2017.30.2.097

Histopathology of leech parasitism on *Capoeta capoeta gracilis*, *Squalius cephalus* and *Carassius auratus*

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Parasitic leeches could directly (through causing poor growth, anemia and wound in the fish) and indirectly (by predisposition of the fish to secondary bacterial and fungal infections) affects their hosts. In the present study, fishes that were attacked by leeches in natural and experimental environment were studied. Pathologic samples were obtained from damages at the site of leech bite, as well as kidney and liver of the fish. Histopathological examination revealed numerous lesions at the site of leech bite including tissue demolition, detachment at the site of leech bite in the epidermis of epithelial tissue in the skin, destructed nucleus in epithelial cells of the skin plus necrosis in the damaged skin and weak inflammatory penetration to acute necrotic damages along with piercing dermis layer. Pathologic lesions in the kidney included some changes such as proliferation by increasing glomerular cells and membrane cells in capillary vein of the kidney, blood cell necrosis in kidney with infiltration of white blood cells mainly mononuclear and less polymorphonuclear which are the symptoms of anemia due to blood feeding and sucking by leeches. There was also a chronic kidney infection probably originated from another part of body such as skin. Moreover, leeches caused hemorrhagic anemia due to blood consumption of the hosts, which led to observation of immature red blood cells. Also results showed that diseases induced by leeched in fish could be acute or chronic, which depends on size of fish, species of leech and severity of infection.

Key words: Parasitic leeches, Histopathology, Skin, Kidney.

Leeches are the only important fish pathogens in the phylum Annelida. Both freshwater and marine leeches are worldwide distributed, infecting various habitats, thereby adversely affecting the fish health. In this context, leeches could serve as vectors of potentially pathogenic organisms including *Trypanosoma, Cryptobia*, haemogregarines and piroplasmas. Moreover, leeches could transmit viruses and bacteria (Woo, 2006). For example, *Piscicola geometra* has been observed to transmit SVC virus to carp (Ahne, 1985). Viral Hemorrhagic Septicemia virus (VHSV) has also isolated from the leech *Myzobdella lugubris* (Faisal and Schulz, 2009). In addition, feeding wounds may become contaminated by opportunistic bacteria and fungi (Kabata, 1985). Additionally, leeches may affect the host through blood consumption. The site of leech bite could also be infected by secondary pathogens (Woo, 2006). Accordingly, knowledge associated with leech fauna of each water resource is essential.

Important fish pathogens effects are usually lo-

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calized and limited to the sites of leech bite on the skin, fins, gills or mouth. The muscular caudal sucker used for attachment usually causes little damage; however, leeches that are semi-permanent parasites may result in a substantial host tissue reaction to the leech bite. Rhynchobdellid leeches feed from host blood or tissue fluid by means of a protrusible proboscis, which is inserted into host tissue, which may lead to localized petechial hemorrhage (Sloan *et al*., 1984; Jones and Woo, 1990). Although the lesion would usually be limited, heavy infestations can cause severe epidermal erosion and even mortality due to large amounts of blood loss or secondary consequences of multiple feeding wounds. Thus, large numbers of leeches in aquaculture facilities should always be considered as a concern for fish health. Fish may tolerate a high number of leeches with little apparent damage, but the lesions would depend on the relative size of the leech compared with the fish (Woo, 2006). Kabata (1985), without providing details, reported that the presence of 100 leeches on a single fish in Africa resulted in no serious harm to the host. Other reports, however, suggest that leeches may be pathogenic or provide portals of entry through their feeding or attachment wounds for secondary pathogens. The high number of leeches would cause weakness and anemia in infected fishes (Kabata, 1985; Paperna, 1996). Nevertheless, the transmission of pathogens by leeches among the other fishes is more important than their feeding-resultant injuries.

The aim of this study is obtaining related information to the Fauna leaches of the Kurdistan province (Iran) water resource and the study of the histopathology of leech parasitism on some fishes. Considering that leeches can be difficult to collect and to identify because they often leave the host after feeding therefore, may go undetected even when abundance is high, and investigation of the characteristics of leeches in the natural environments might be impossible. Hence, an experimental study was designed to address whether the identified leeches are parasitic or saprophytic, how the leeches attack the fish, whether a specific characteristics or special part of the host is preferred by leech and the process and rate of imposing lesions to the host.

Materials and methods

Sampling methods

As leeches are usually located under the rocks in the water, we can find them at the plants and trees logs. Sampling of side rocks of river and also inner sides of river was performed by forceps. In this regard, the rocks and cobble stones of river-side or the other sides that had the slower flow of water were separated by forceps in order to find leeches. Most of the leeches were stuck at the bottom of rocks and submerged plant floating in the water or even at the trashes in the river. The samples were also collected from the bottom of turtles or the preyed fish.

Five sampling stations were chosen in Kurdistan water resources. In each sampling station, geographical characteristics of sites of leech collection were registered using GPS system. After putting the leeches to sample containers, the leeches were sedated using ethanol 5-10% (preventing leeches' body contraction), then the leeches were fixed by formalin 10%, which were consequently transferred to laboratory. Afterwards, the leeches were stained using Carmen acetic acid for further assessment. Identification of the leeches species was performed as previously described (Sawyer, 1986 (a, b); Klemm, 2001; Nesemann, 1999; Elliott & Mann, 1987 and Gussev, 1979).

Experimental leech pollution of fishes

Leeches were kept in aquarium covered by a black plastic for darkening the environment due to photophobia of leeches. The aquarium was filled with stone and sand, and equipped with proper ventilation and water flow, resembling the natural condition of leeches. Prior to transfer to the aquarium, its temper-

ature was equilibrated to avoid stress to leeches. Leeches were maintained for 16 days in the aquarium for acclimation to the condition. Next, goldfish (*Carassius auratus*) were transferred to the aquarium. Fish, which were found with attached leeches, were subjected to histopathological examination of site of leech bite, liver and kidney tissue as aforementioned.

Histopathological examination

Site of leech bite on skin, liver and kidney specimens were harvested from afflicted fish. The specimens were fixed in 10% phosphate-buffered formalin solution. Then the specimens were embedded in paraffin and processed routinely for histopathological evaluation of tissues. Sections were stained with haematoxylin and eosin (H&E).

Results

Seven species of leeches, which were isolated in the present study, had been isolated and identified previously from water resources of Kurdistan Province in Iran (Salimi *et al*., 2011), including *Helobdella stagnalis*, *Placobdella costata*, *Hemiclepsis marginata*, *Erpobdella lineate*, *Erpobdella ocloculata*, *Hirudo medicinalis*, *Dina lineate lineata.* Among recognized species in Kurdistan Province water resource, *P. costata* and *H. marginata* had attached to *Capoeta capoeta gracilis* (Fig. 1) and *Squalius cephalus*.

Gross features

Also five days after transferring fishes to the aquarium containing leeches some sticking samples of *H. medicinalis* and *H. marginata* to the golden carp were observed. Following attachment to fish, fish were began to shed scale dropping at the site of leech bite, leading to restlessness in fish in order to separate the leech. Finally, the leeches pierced the skin completely after 36 hours and fed from fish blood and liquid tissues. After the fish death, leeches left them (Fig. 2). If their corps remained in the aquarium, *H. stagnalis* and *E. octoculata* leeches would start feeding from them.

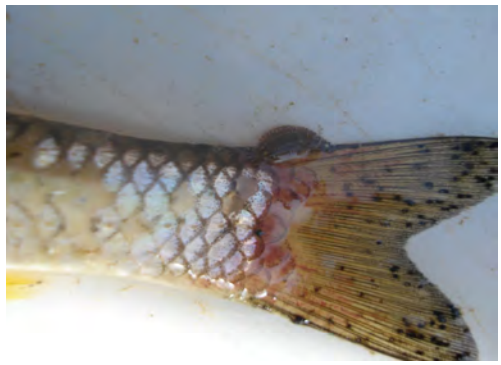

Fig. 1. The leeches (*Hemiclepsis marginata)* sticking to the *Capoeta capoeta gracilis*.

Fig. 2. The leech (*Hirudo medicinalis)* sticking to the goldfish (*Carassius auratus*).

In all the observed cases, *H. medicinalis* were attached to abdomen of the fish and caused death in fish short after attachment, whereas *H. marginata* either in natural or experimental condition chose skin and fins to attachment and mortality rate was considerably lower in the respective fish, in such cases, death occurred long after leech attachment. *P. costata*, which had been detected from fish harvested from water resources, was merely attached to fins.

Histopathology features

Skin (naturally and experimentally infected fish)

The following histopathological signs in skin tissue damages were observed in all the preyed fishes espe-

cially those that were leeches stuck to them and experimental infection of the leech. Tissue destruction and break were observed in the place of leeches to the epidermis of epithelial tissue in the skin (Fig. 3). Because of traumatic damages of the leeches stick to the skin in the connection place of leeches the color changes have occurred in skin melanocytes along with becoming thick in the cuticle layer and elevation in Malpighi cells (Fig. 4). There were also some signs of inflammation response along with inflammatory cells mainly in the sort of lymphocytes (L) in epidermis of damaged skin. In damaged cells necrosis states as nucleus divides in to different pieces sometimes its completed absence (Fig. 5). The type of damages as the chronic inflammation along with the penetration of lymphocytic inflammation cells were observed. There were some colored melanosomes (melanomacrophage centers) in the dermis region and some changes in the shape of status spongiosus of skin in the epidermis layer (Fig. 6). Color changes sometimes as the gray-blue glows because of the necrosis of collagen groups in the skin.

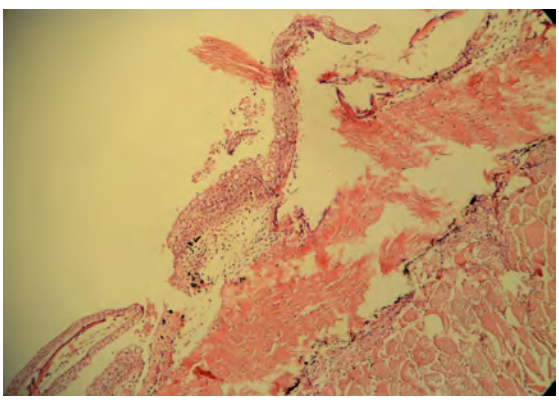

Fig. 3. The destruction of epiderm layer of epithelial tissue in the connection place of leeches (H&E stain, magnifying 100).

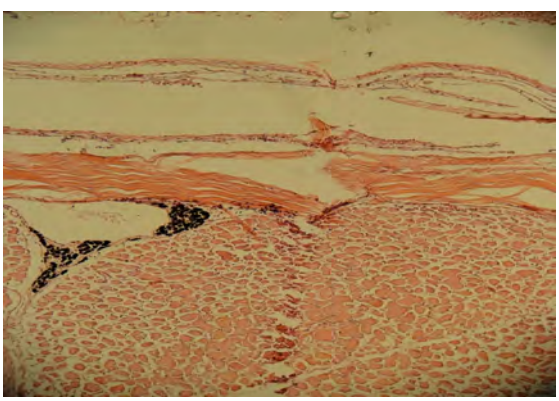

Fig. 4. The destruction of epiderm layer along with color changes in skin melanocyte (H&E stain, magnifying 100).

Kidney (naturally infected fish)

All the preyed fishes especially those that were

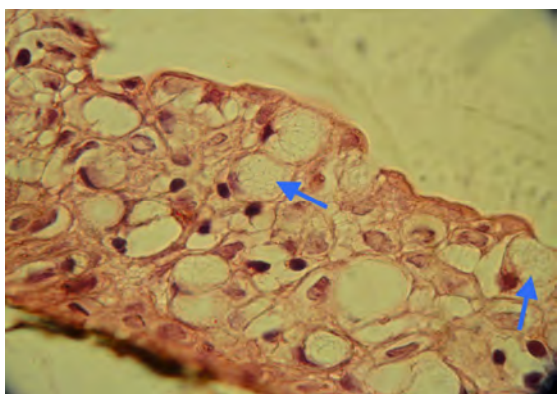

Fig. 5. The layers destructed in epiderm along with the observation of necrosis (H&E stain, magnify 1000).

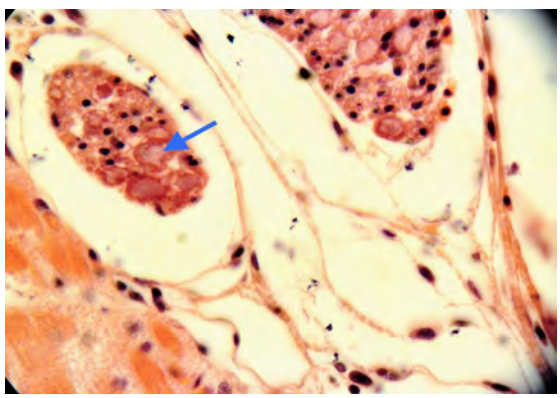

Fig. 6. The observation of colored melanosomes (melanomacrophage centers) in the dermis region, maybe it's because of parasites stimulation on melanophores and the change as the status spongiosus of skin. (H&E stain, magnify 1000).

leeches stuck to them are investigated and following kidney histopathological signs were observed:

Observation of necrosis cells in tubules and the increase of the number of melanomacrophage centers in kidney can be a probable sign of chronic infection caused by parasite (Fig. 7). There were also glomerulonephritis state as the increase of thickness in membrane cells of Glomerulus and the attack of inflammatory cells among kidney and Tubules (nephrons). Hemorrhagic anemia and bleeding because of blood consuming of host by leeches caused to lose RBC. (In this state we can see immature red blood cells in the blood) (Fig. 8). Also the proliferative change

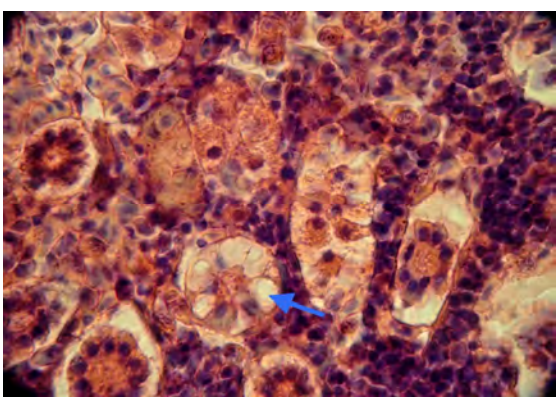

Fig. 7. Tubular necrosis and the increase of melanomacrophage centers in kidney. (H&E stain, magnify 1000).

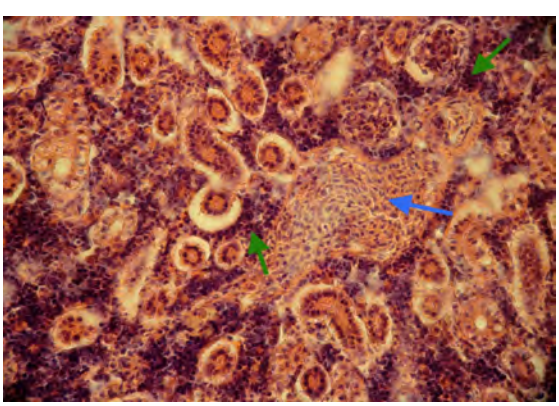

Fig. 8. The increase of inflammatory cells (green arrows) and hemorrhagic in the kidney (blue arrow) (H&E stain, magnify 400).

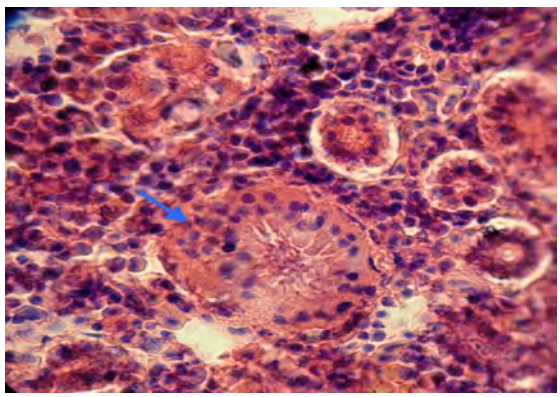

Fig. 9. The observation of proliferative change as the increasing glomeruli cells and also increasing membrane cells in capillary vessel. (H&E stain, magnify 1000).

were observed like the kidney proliferative disease as the increasing glomeruli cells and also increasing membrane cells in capillary vessel (Fig. 9). Observation of necrosis cells kidney blood maker tissue a long with increasing the penetration of withe blood cells mainly mononuclear (lymphocyte) and less polymorphonuclear (N) Which are some signs of disorders caused by anemia because of feeding and blood suck by leeches and also a kidney chronic infection that its initial source is somewhere else like skin.

Liver (naturally and experimentally infected fish)

In pathologic studies of liver, no change due to the pollution by leeches was observed.

Discussion

Leeches alone are generally not considered important fish pathogens. Effects are usually localized and restricted to attachment and/or feeding sites on the skin, fins, gills or mouth (Sloan et al., 1984; Jones and Woo, 1990). Even though pathology is usually localized, heavy infestations can result in severe epidermal erosion and even mortality because of large amounts of blood loss or secondary effects of multiple feeding wounds. Thus, large numbers of leeches in aquaculture facilities should always be a cause for

concern. Fish can tolerate a high burden of leeches with little apparent effect, but pathology may depend on the relative size of the leech compared with the fish. One of the earliest accounts of a leech causing mortality is the report by Badham (1916) involving the leech *Austrobdella translucens* and the sand whiting, *Sillago ciliata*, in Australia. A few dozen whiting were periodically stocked with other species in a saltwater pond over a number of years and on each occasion the whiting were killed by the leeches.

Paperna and Zwerner (1974) reported removing over 500 *Myzobdella lugubris* from a single moribund white catfish (*Ictalurus catu*) in the York River estuary in Virginia, USA. Leeches were in the mouth, under the operculum, on the skin fold behind the lower jaw and at the bases of the fins. Extensive histopathological changes caused by the leech included inflammation, displacement and erosion of the dermis and hyperplasia of the epithelium. All pathological changes were attributed to leeches and it was concluded that leeches were at least a major contributing factor to the distressed condition of the fish .

Heavy leech infections have variable effects on fish hosts. In Lake Victoria, 19% of all studied *Bagrus docmac* harbored leeches, with a mean number of 26 per infected fish. Leeches were attached to the external perimeters of the mouth region and pathological changes were limited to bite signs and mild tissue changes even in the most heavily infected individuals (harbouring over 100 leeches). More severe damage was evident in piscicolid infection inside the mouth (mainly on the palate and the corners of the jaws) of grey mullets. Infections occurred in several mullet species (in almost all examined *L. dummurelli* and *L. tricuspidata*, and occasionally also in *Liza richardsoni*, and *Mugil cephalus*) but only in mature fish (over 200 mm in length). The number of leeches per fish was variable and could reach up to 67. Lesions only partially corresponded to observed infection loads and developed in the mouth in the area with the highest predilection for leech attachment, i.e. in the anterior part of the mouth roof and the articulation zone of the lower and upper jaw. Damage to skin comprised of bite wounds, hemorrhages and erosion of the mucous membranes. In heavy infections, leeches were also attached in the craters of the deeper lesions. In some heavy infections the gular membrane also became perforated (Paperna, 1996).

Natural hyper infection in an American catfish (*Ictalurus catus*), isolated in the USA, induced epithelial hyperplasia plus inflammatory changes and hemorrhages in the dermis (Paperna and Zwerner, 1974). Fish confined to ponds seem to be more vulnerable to leech attack. Heavy leech infection of eyes and nostrils of cultured carp, which resulted in mortalities, have been reported from Ghana (Ghana Ministry of Agriculture, 1965). In Iraq, *H. marginata* infection coincided with incidence of mortality in pond-reared carp (Khalifa, 1985). Leech attacks were reported in Clarias spp. and tilapia in Southeast Asia. The leeches involved were unidentified local piscicolids or Glossiphoniids which induced lesions similar to that reported above (Kabata, 1985). Leeches have recently been implicated in pathology of tank-reared orange-spotted grouper, Epinephelus coioides, in the Philippines. Heavy infestations of Zeylanicobdella arugamensis occurred in multiple patches of several hundred leeches on and at the bases of the fins and in the skin folds of the lower jaw. The affected fish had multiple areas of hemorrhage and hyperplasia at attachment and feeding sites (Cruz-Lacierda et al., 2000), but there was no report of mortality.

In Japan in Yodo River, the pollution of *Carassius auratus Langsdorfii* and *C. cuvieri* has been observed in 2007. These leeches mainly covered operculum region of the fishes where bleeding and destruction were observed (Ogawa and Rusink, 2007).

The histopathology of leech, *Austrobdella bilobata*, infestation on the upper palate and pelvic fin of the yellow fin bream, *Acanthopagrus australis*, is described. The feeding site was often hemorrhagic and swelling surrounded the attachment site. Histological

examination revealed massive cellular infiltration (predominantly lymphocyte and macrophage) in the submucosa beneath the feeding and attachment sites. The latter also had a complete erosion of the integument beneath the parasite. Localized proliferation of connective tissue fibers and zones of necrosis and edema were present in some cases (Roubal, 1986).

In all the studies, only infections with high numbers of leeches led to mortality; nevertheless, the relationship between the fish size and number of leeches should be considered. The results showed that even infection with one *H. medicinalis* could result in death in short period of time in fingerling goldfish. There is no report indicating the attack of *H. medicinalis*; however, the present study revealed that this species could attack fish and be one of the most detrimental species for fish. The results showed that the mortality rate was related to attachment site of the leech and the mortality rate would be higher in case of leech attachment to abdomen as compared with attachment to other parts of the body including fins. *H. medicinalis* choose fish abdomen for attachment, which could have been resulted from the specific anatomical characteristic and type feeding of the leech, and considering the loss of vast blood and biological fluids over a short period of time, it could cause high mortality in fish. Absence of any report for the infection with *H. medicinalis* in natural condition could originate from the fact that infection with this species would shortly lead to death. Given that this species is produced all over the world for medical purposes, swage system of leech farms should be under control and built far from the fish hatcheries. Since leeches invaded the hatchery through the freshwater supply from a nearby stream and attacked fry as they hatched in trays. Once attached to the fry, leeches quickly became gorged with blood and the fry invariably died, apparently from blood loss. Therefore, presence of this species should be considered as a threat for hatcheries and as a cause of reduced survival rate.

Based on the result, the sticking region of leeches to the fish, was accompanied by scale dropping, destructing mucous membrane, and bleeding. Due to traumatic damages, colored melanocytes resulted from parasitic stimulation on melanophores, were observable in dermis. Also, colored changes were sometimes observed in the form of gray-blue glows due to collagen clusters necrosis. There was cellular hyperplasia in epithelial tissue, which probably resulted from the decrease of water temperature, according to the water temperature of investigated region. In generated inflammatory response, inflammatory cells are mostly lymphocytes. Also, the wound place caused by leeches, became an appropriate center for getting secondary bacterial and fungus infection and saprophyte infections. It's necessary to mention that identifying bacteria, fungi and other factors needs to be studied more.

In the kidneys, pathologic damages were observed in the form of proliferation which resembled proliferative kidney disease. This damage is observed in the form of Glomerular cells and membrane cells increase in capillary vessel. Other observed damages consisted of necrosis in kidney tissue and chronic infection. This chronic infection was accompanied by increasing white-blood cells penetration, mostly mononuclear and less polymorphonuclear. The origin of this infection could be another part of body like skin. Finally, hemorrhagic anemia was seen due to blood consuming by leeches causing blood loss leading to observation of immature red blood cells in environmental blood. In liver, no damage related to the pollution by leeches was observed. To, our knowledge, there is no report describing leech-caused pathologic lesions in fish kidney and liver, and the present report is probably presented for the first time.

In histopathological examinations in naturally and experimentally infected fish, it was revealed that the larger fish and the lower number of leeches, the slower development of pathologic lesions and the consequences of hematophagy in internal organs would be; indeed, the species of leech and the site of attach-

ment are the substantial determinant factors in this regard. In goldfish infected with *H. medicinalis* no considerable lesion were observed in the abdominal cavity except for pale liver and small spleen; in addition, the gills were obviously pale. Mortality of these fish was resulted from severe hemorrhage. However, renal histopathologic lesions in fish infected with *H. marginata* and *P. costata* were observed as mentioned in the result part, indicating a chronic disease characteristic especially in kidney. Therefore, it could be concluded that diseases induced by leeched in fish could be acute or chronic, which depends on size of fish, species of leech and severity of infection.

Acknowledgement

The present study was supported by Islamic Azad University, Sanandaj Branch, Iran. We thank Investigation and Management Office of Aquatic Animal Health, Veterinary Organization of Iran.

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Manuscript Received : Aug 3, 2015
Revised : Mar 23, 2016
Accepted : May 30, 2016