Global Veterinaria 3 (2): 113-119, 2009
ISSN 1992-6197

Investigations on White Spots Disease (*Ichthyophthriasis*) in Catfish (*Clarias gariepinus*) with Special Reference To the Immune Response

[1]H.A.M. Osman, [2]Laya F. El-Bana, [1]A.E. Noor El Deen and [1]O.K. Abd El-Hady

[1]Department of Hydrobiology, National Research Center, Dokki, Giza, Egypt
[2]Educational Veterinary Hospital, Faculty of Veterinary Medicine, Zgazig University,

Abstract: The experiment was carried out on 90 apparently healthy parasite free 80-100 g catfish (*Clarias gariepinus*), used for determining the immune response of ich infected fish, they were divided into 6 groups of 15 fish each, 1st group is naïve fish (control negative), the 2nd group I/P injected with excised skin culture fluids from infected & treated fish, the 3rd group I/P injected with live theronts, the 4th group I/P injected with killed theronts, 5th group was infected and treated group from Gyrodactylus sp. (after 2 weeks pt) and the 6th group was treated group from Ich with formalin (after 2 weeks pt) 5000 theronts/fish placed with each group for challenge for 5 days, clinical signs, morbidity, mortality, density of infection with Ich and antibody titre was recorded for 8 weeks. Antibody titre was significantly higher in infected and treated fish then followed by I/P injection of live theronts then killed theronts then which I/P injected with excised skin culture then non specific immunized fish and the lowest antibody titre was recorded in (control – ve) naïve fish.

Key words: Catfish · Ich · Theronts · Excised skin culture · Antibody titre

INTRODUCTION

White spots (Ichthyophthiriasis) is a prevalent ectoparasitic disease, mostly affecting cultured and aquarium fishes. The morbidity rate due to this disease may reach up to 100%, causing great economic losses in fish farms. Ichthyophthiriasis has also been known as sand grain, gravel or ich disease [1-4]. This dangerous ectoparasite mainly attacks skin fins, gills and buccal cavity and is characterized by the presence of white spots all over the external body surface. *Ichthyophthrius mulitifclis* has a simple, direct life cycle, divided into three distinct stages. The trophont resides and feeds in the epidermis of the host where it can obtain a diameter of up to one mm., the mature trophont escape form the epidermis to the surroundings freshwater, where some of the parasites settle and develop into encysted tomonts. In this tomontocyst stage numerous daughter cells (tomites) are produced the number of tomites resulting from one tomont varies between 50 and a few thousands [5]. These stages escape the cyst as so called theronts (length 20-60 mm) ready to infect the fish epithelium. A number of studies have shown that all these life cycle stages are extremely temperature dependant. 18 - 25 °C [5]. A number of studies have indicated that sublethal infections in the host are able to induce an aquried resistance against re – infection [6-8]. The protective mechanisms responsible for host resistance not been fully elucidated, however, [9] observations suggest that immuno prophylactic measures should be considered in order to control Ich infection, one of these possibilities could be vaccination with Ich preparations [10-12]. Antibodies against antigens of Ich were found in both serum and mucous collected from Ich immune fish [13,14]. Some reports showed that serum antibody levels in fish did not correlate with induced protection after immunization [15]. So, these results suggest that there may be other protective mechanisms against these pathogens in addition to the systemic immune response, Coetaneous antibodies have been found to be involved in protection against pathogens [16]. Trials [17] were carried out for collection of trophonts from infected fish and subsequent production of tomonts and theronts for vaccine. Applying this method resulted in a clear protection against re – infection and also activated host factors following treatment of infection with skin parasitic monogeneans (*Gyrodactylus derjavini*). Also this method was reported to reduce the establishment of Ich in

Correspondign Author: Dr. Husein A. Osman, Department of Hydrobiology, National Research Centre, Postal code: 12622, Dokki, Giza, Egypt

Rainbow trout [8,18]. Therefore the present study was carried out to determine the cause of immune response of infected catfish (*Clarias gariepinus*) following infection and reinfection with Ich, whereas few publications have addressed the problem of immunity, especially the acquired type in wild fish populations.

MATERIAL AND METHODS

Experimental Fish: A total number of 90 apparently healthy parasite free (examined) catfish (80-100 g weight) were obtained alive from private fish farm at Kafr El Sheikh governorate. The fish were kept in well aerated chlorine free tap water at 25°C.

Primary Exposure to Infection: All fish were infected with *I. multifilus via* exposure to a high dose of collected theronts (approximately 5000 / fish) using the immersion method as described by McCallum [19]. Following exposure for 5 hours in the dark, fish were transferred to glass aquarium supplied with aerated dechlorinated tap water and kept for five days at 20°C.

Treatment Trial of Ich Infected Fish: Naturally heavy Ich infected were treated with formalin at a concentration of 100 mlL^{-1} for 1 hour to kill theronts and prevent re infection, the fish were treated daily for 7 days until no white spot were seen on skin and gills of the fish. The aquarium with uninfected fish were used as control and had the same treatment as infected aquarium.

Collection of *Ichthyophthirius multifilus* Tomonts: Fish with natural heavy parasitic infection (5days post-infection) were anesthetized with tricaine methane sulfonate (MS 222 150 mg/l) then rinsed with tap water and the skin was gently scraped to dislodge the tomonts. The isolated tomonts were put on a 250 µm mesh sieve to retain fish skin and mucus, then put over a second 75 µm mesh sieve and flushed with running water. The trapped tomonts over the 75 µm mesh sieve were collected and washed with water using wash bottle, then transferred into one – liter glass beaker containing 500 ml water as a modification of the method of Noe and Dickerson [20].

Obtaining of *Ichthyophthirius multifiliis* Live Ther Onts: The traped tomonts over the 75 µm mesh sieve were collected and incubated at 24±1°C for two hours, then the water was decanted to get rid of other contaminants and 100 ml tap water was added to the beaker and incubated at 24±1°C for 20-24 hours for development of therants. Developed theronts were filtrated through 38 µm mesh sieve and examined under binuclear microscope for viability [21]. Theronts were counted with a sedgwickrafter cell (VWR Scientific products, Atlanta, GA, USA). Live theronts were prepared for IP injection after concentration by centrifugation at 500 xg for 5min., collected and subdivided into 2 parts, one was killed by adding formalin, washed and diluted with sterile saline solution,while the other part was used as it is (live) after it was diluted with sterile saline according to Burkart *et a*l.[17].

Collection of Culture Fluid from Skin of Immumized Fish: Ten treated fish were used for collection of culture fluid from skin according to Xu *et al*.[3]. In brief,, fish were kept for 15 min in steril water to flush loosly bound bacteria. After fish were killed by pithing the brain, the skin was collected from lateral body wall with sterile instruments and cut into 5 x 5 mm pieces. The excised tissues were washed three times with Hank's balanced salt solution and with medium 199 (Sigma), approximately 900 mg of excised skin and 3ml of culture medium was added to each well of a 6 – well plate.The culture fluids was collected after 24 hrs incubation in dark at 20°C then the culture fluids were centrifuged at 228 xg for 10 min., the supernatant was scaned and preserved till use.

Preparation of Catfish Treated from Gyrodactylus: Twenty natural infected catfish with gyrodactylus were treated with praziquantel bath for 15 min [22] then *in vitro* transferred to clean arearted free chlorine tap water. After 12 hrs, fish were retreated again from gyrodactylus with praziquantel bath for 15 min. Fish were examined after each treatment with skin scraping for detection of gyrodactylus under disecting microscope.

Blood Sampling and Antibody Titre Measuring: Five fish per each immunized group were removed and blood samples were collected (caudal vein) to determine the antibody level against Ich. at 30 days post – immunization. Fish were anesthetized with 150 mg/l MS 222 prior to blood sampling. Blood was allowed to coagulate at 4°C overnight and then centrifuged at 6000 xg for 5 min., sera were aspirated, collected into microcentrifuge tubes and stored at 20°C. antibodies against Ich in serum were measured using the theront immobllization assay [23]. Theront mobility was determined with an inverted microscope and immobilization titer was defined as the highest dilution in which all theronts lost mobility and aggregated.

Experimental Design and Challenge: Six groups of 15 fish each [naïve catfish *(clarias gariepinus*), 80-100 g in weight were used.

The 1st group is control negative, the 2nd group is I/P injected with excised skin culture fliuds 0.5 ml from immune fish, the3rd group I/P injected with live theronts, 0.5 ml, the 4th group(the treated group from gyrodactylus after 2 weeks), I/P injected with killed theronts with formalin 0.5 ml,, the5th group was the treated group from Ich with formalin. with 5000 theronts/fish was placed with each group in experimental aquaria for 5 days, 2 weeks post treatment.

Then the fish of each group were transferred to another aquaria without theronts and monitered for the clinical sings, morbdity, mortality and density of parasitic infection were recorded. Also calculation of antibody titre for each group at the end of the fourth week was carried out according to Hai *et al*. [24].

The 1st and 5th group injected I/P with steril saline solution. The relative percent survival (RPS) for each group was calculated [25] as:

$$RPS = 1 - \frac{\text{Mortality \% of vaccinated}}{\text{Mortality \% of control}}$$

Statistical Analysis: Antibody levels against Ich in different immunized groups were compared with Duncan's multiple range test at probabilities of < 0.05 which was.

RESULTS

Result and Identification of Ich from Natural Diseased Catfish: The clinical signs and behavioral change of heavy naturally infected catfish with white spot revealed the presence of large number of white spots all over the body surface and fins, there was increased mucus production from all the body. Infected fishes swim rapidly and rub their bodies against sides of aquarium as the disease progress, the fish gasp for oxygen and become increasingly lethargic and eventually stop feeding. Experimentally infected catfish *Clarias gariepinus* showed the same clinical signs of naturally infected fish.

Examination of wet mounts of disloged parasites from skin and gills revealed oval to round shap large ciliated parasites (trophont) with horseshoe – shaped or sausage shape macronucleus (plate 1C and plate 1D) which is pathognomic sign of Ich multifilus infection.

The density of infection, 8 weeks after experimental challenge was maximum in the control group, followed by non specific immune response then excised skin culture, then killed trophont then live trophont and the lowest

Table 1: Density of infection with Ich and survival of catfish (*clarias gariepnus*) challenged with 5.000 theront/fish

Time	Control (naive)		Excised skin cultur		Live trophont		Killed trophont		Non specific immun		Infected, formalintreated	
in week	Dens. Inf.	Surviv.	Dens. Inf.	Surviv.	Dens. Inf.	Surviv.	Dens. Inf.	Surviv.	Dens. Inf.	Surviv.	Dens. Inf.	surviv
1	+++	12	++	15	++	15	++	15	++	15	+	15
2	+++	9	++	15	+	15	+++	15	++	15	-	15
3	+++	8	+	15	+	15	+++	14	++	13	-	15
4	+++	8	+	15	-	15	++	14	++	11	-	15
5	+++	7	-	15	-	15	+	13	+++	10	-	15
6	++	5	-	15	-	15	-	11	++	9	-	15
7	++	3	-	14	-	14	-	10	++	6	-	15
8	++	3	-	13	-	14	-	9	++	5	-	15

Table 2: Antibody titer (Mean + SE) and relative percent survival of catfish *Clarias gariepinus* after 4 weeks of challenge

Treatment	Number of fish	Antibody titer	mortality	% of mortality	RPS relative percent survival
Control (naïve fish)	15	c37 ± 2.7	12	80	0
Excised skin culture	15	b433 ± 30.40	6	40	0.5
Live theronts	15	a1801 ± 144.80	1	6.7	0.92
Killed theronts	15	a1127 ± 90.16	2	13.3	0.83
Non specific immun	15	b290 ± 23.20	10	66.6	0.17
infected formalin treatment	15	a1952 ± 156.16	0	0	1

Immobilization titer was the reciprocal of the highest dilution in which all theronts were immobilized, each value is the mean of five samples within a column, means with different superscript are significantly different (P<0.05)

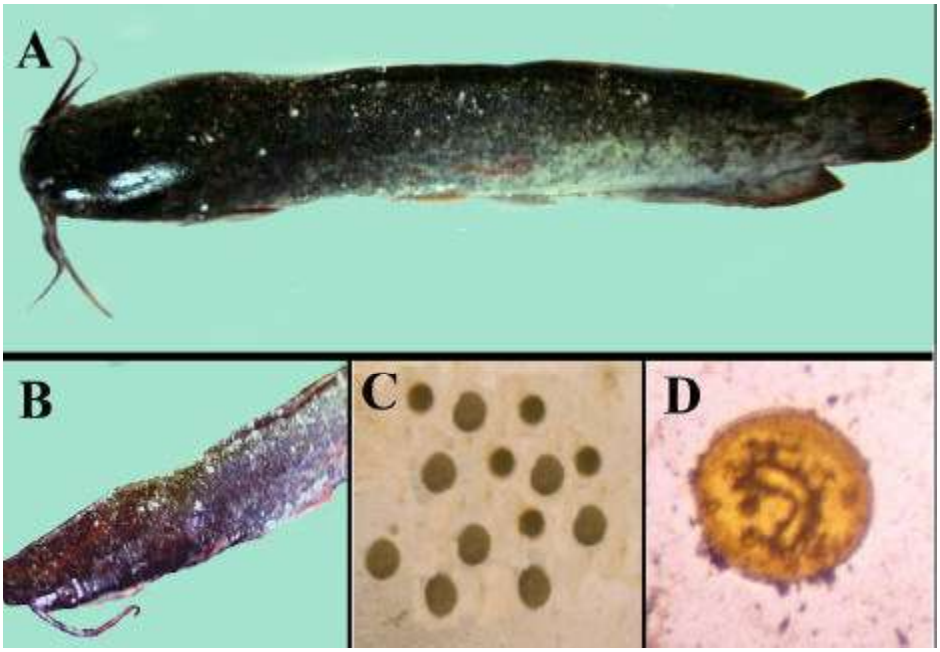

Plate 1: A: catfish *Claris gariepinus* showing small numbers of white spots on the body surface (light infestation +). B: catfish *Claris gariepinus* showing large numbers of white spots on the head and trunk (heavy infestation +++). C: wet mount showing large number of *Ichthyophthirius multifiliis* trophonts in skin preparation (40X). D: wet mount showing *Ichthyophthirius multifiliis* trophonts with macronucleus (100X).

density of infection was in infected formaline treated group (Table 1). The highest antibody titer was recorded in infected - formalin treated group then after injected of live trophont I/P followed by killed trophont with formalin then excised skin culture and the lowest antibody titer was recorded in fish infected and treated with gyrodactylides (non specfic immune response) the mortality percent was highest in the control group while the lowest mortality rate was recorded in fish with history of infection and treated with formalin. The highest RPS was determined in the infected group treated with formalin, while it was zero in the control group (Table 2).

The calculated antibody titer for different groups was correlated with the mortality rate and denisty of infection Immobilization titer was the reciprocal of the highest dilution in which all theronts were immobilized, each value is the mean of five samples within a column, means with different superscript are significantly different ($P< 0.05$).

DISCUSSION

The clinical signs recorded in this study due to *Ich multifilus* infection (white spot disease) are coincide with those recorded by most researchers as [21, 26-30,]. The results of wet mounts of dislodged examination under microscope revealed oval to round shape ciliated parasites (trophont) stage with its characteristic sausage or horseshoe – shaped macronucleus these findings were coincide with those recorded by most researchers [21,29-32]. *Ich mulftifilus* infection was maintained in the laboratory using naïve fish because *in vitro* cultivation method for this protozoan still under development Infection was maintained for about 2 month under laboratory conditions, this coincide with routine method [33-35]. The current trials extend the growth period of *Ich multifiliis* trophonts at catfish (*Clarias gariepinus*) in the laboratory succeeded to sustain its growth for 30 days at temperature of 8°C. These results coincide to the extent with those reported by Noe and Dickerson [20]. The minimal infective dose of *Ich multifilus* theronts to channel catfish under laboratory conditions was determined to be 1000 theronts / fish [21]. However, in the present study, challenge was carried out with about 5000 theronts/ fish for 5 days to be sure that the infection must occur.

The present study revealed that the infected and formaline treated catfish have the highest antibody titer and this result nearly agree with the result of Eissa [14], who reported that the sublethal infection provide the fish with a protection against re-infection, the cause of this

protection was still unkwon. Ich infection in carp demonstrated a protective response dependent upon the primary infection which induce strong immune response [8]. The last authors reported that the experimental infections of rainbow trout resulting in parasite loads of up to approximately 65 trophont per fish which provided the fish with complete protection compared to control fish. On the other hand, the present study demonstrated the antibody titre of gyrodactylids infected and treated catfish (non specific) was lowest e than all other groups except the control group. These results were confirmed by Buchmann *et al.*[18] who found that the activation of the responses against other parasite not induce some protection against Ich. So immunization with gyrodactylus seems to protect the fish partially against Ich. On the other hand [10,18] showed a high degree of cross – reactivity in gold fish immunized with ciliate *tetrahymena pyriformis* by immersion or Ip injection, they added that the resultant protection was not only against Ich. but also against other ectoparasites like *Ichthyobda necator* and chilodonella. The study revealed that such methods are likely to be less effective than specific immunization against Ich. (Ip killed theronts or live thronts). *In vitro* assays of the non – specific immune response suggested that the complement action could explain immobilization and lysis of Ich theronts in non – immune fish serum [8,18]. Thus the complement in the serum could take part in immune response of naïve fish. This observations do not exclude that there are a number of other non – specific humeral elements in fish bodies which play a role in protective processes. The antibody titer of fish immunized with skin culture was higher than that of non specific immune response and higher than the control group, this indicate that the skin take considerable part in protection against Ich. infection in catfish. *T*his result was previously obtained and confirmed by used histopathological examination which illustrated epithelial hyperplasia and extensive proliferation of mucous cells in the affected area, in association with cellular aggregations around the parasite at the site of infection[36,37]. Moreover, [8] noticed that exposure of brown trout fry to Ich. infection is associated with marked degranulation of thionin – positive cells (putative mast cells) in fish skin. Other studies have clearly indicated that humeral factors are associated with immunity in fish skin against Ich. in the same time, [13] indicated that the antibodies in fish serum against Ich. Are able to agglutinate trophonts *in vitro* by adding serum or mucous from immunized fish. This confirmed the role of skin in protection process.

The present study revealed that the IP injection of live theronts induced revealed the highest antibody titer followed by IP injection of killed theronts to catfish. *T*his result coincide with those of Burkart *et al*. [17] who immunized channel catfish with killed trophonts or theronts and recorded clear protection against infection. The access to sufficient parasite material for production of vaccine is hampered by difficulties of cultivating Ich. and a full *in vitro* life cycle is needed to obtain sufficient parasite material for vaccine production.

Vaccine production has been achieved depending on collection of trophonts from infected fish and subsequent production of tomonts and theronts. This was achieved in this study where collection and dislodge of tomont from heavy infected catfish was carried out by filtration and incubation the tomont to obtain theronts for IP, In this respect, [10] evaluated a number of vaccine candidates including live tomites when injected IP into gold fish, obtained a high degree of protection as 97.8% uninfected fish after challenge compared with 2.2% uninfected in control group. Also [12] injected rainbow traut fry IP with homogenate of formalin killed trophonts and obtained a significant reduction in infection intensity in immunized fry compared to naïve fish.

It can be concluded that immune response of freshwater fish to infections with Ich. Is one of the best characterized immuno – parasitology in fish. Specific and non specific immune effector arms have both been shown to contribute response in Ich infection humoral and cellular elements in the immune response have been identified to interact with parasite molecules. Strong elements of non specific factors in host fish should be considered as a possible way of inducing protection to large number of fish, finally there is does not mean that there is a full consensus about the exact immune mechanisms involved in the fish resistance against infection of Ich. Thus the immune response protection against infection of Ich is a complex process of all these immune mechanisms.

REFERENCES

1. Richards, J. and T.E. McDonald, 1998. *Ichthyophthirius multifiliis* (Ich) epizootics in Spawning Sockeye Salmon in British Columbia, Canada. J. Aquatic. Animal. Health, 10: 143-151.
2. Traxler, G.S., J., Richards and T.E. McDonald, 1998. *Ichthyophthirius multifiliis* (Ich) epizootics in Spawning Sockeye Salmon in British Columbia, Canadian J. Aquat. Animal Health, 10: 143-151.

3. Xu, D., P.H. Klesius, C.A. Shoemaker and J.J. Evans, 2002. The early development of *Ichthyophthirius multifiliis* in Channel Catfish inVitro. J. Aquatic Animal Health, 12(4): 290-296.
4. Eissa, I.A.M., 2004. Parasitic fish diseases in Egypt Chapter one pp: 4.
5. Li, A. and K. Buchmann, 2001. Temperature dependent and salinity dependent development of a Nordic strain of *Ichthyophthirius multifiliis* from rainbow trout. J. Applied. Ichthyology., in press.
6. Clark, T.G. and H.W. Dickerson, 1997. Antibody-mediated effects on parasite behavior: evidence of a novel mechanism of immunity against a parasitic protist. Parasitology. Today, 13: 477-480.
7. Gleeson, D.J., H.I. McCallum and I.P.F. Owens, 2000. Differences in initial and acquired resistance to *Ichthyophthirius multifiliis* between populations of rainbowfish. J. Fish Biology, 57: 466-475.
8. Sigh, J. and K. Buchmann, 2001a. Comparison of immobilization assays and enzyme linked immunosorbent assays for detection of rainbow trout antibody titres against *Ichthyophthirius multifiliis* Fouquet, 1876. J. Fish Dis., 24: 49-51.
9. Cross, M.L. and R.A. Matthews, 1992. Ichthyophthiriasis in carp, *Cyprinus carpio* L.: fate of parasites in immunized fish. J. Fish Dis., 15: 497-505.
10. Ling, K.H., Y.M. Sin and T.J. Lam, 1993. Protection of goldfish against some common ectoparasitic protozoans using *Ichthyophthirius multifiliis* and *Tetrahymena* for vaccination. Aquaculture, 116: 303-314.
11. He, J., Z. Yin, G. Xu, Z. Gong, T.J. Lam and Y.M. Sin, 1997. Protection of goldfish against *Ichthyophthirius multifiliis* by immunization with a recombinant vaccine. Aquaculture, 158: 1-10.
12. Dalgaard, M., A. Li and K. Buchmann, 2001. Immunisation of rainbow trout fry with *Ichthyophthirius multifiliis* sonicate: protection of host and immunological changes. (submitted).
13. Wahli, T. and W. Meier, 1985. Ichthyophthiriasis in trout: Investigation of natural defence mechanisms. In: Ellis, A.E. (Ed.), Fish and Shellfish Pathology. Academic Press, London, pp: 347-352.
14. Houghton, G. and R.A. Matthews, 1990. Immunosuppression in, juvenile carp,Cyprinus carpio (L.),: the effects of the corticosteroid triamcinolone acetonide and hydrocortisone 21 hemisuccinate (cortisol) on acquired immunity and the humoral antibody response to Ichthyophthirius multifliis Fouquet. J. Fish. Diseases, 13: 269-280.
15. Clark, T.G., H.W. Dickerson and R.C. Findly, 1988. Immune response of channel catfish to ciliary antigens of Ichthyophthirius multifliis. Developmenal. Comparative. Immunology, 12: 203-208.
16. Lin, T.I., T.G. Clark and H. Dickerson, 1996. Passive immunization of channel catfish (*Ictalurus punctatus*) against the ciliated protozoan parasine *Ichthyophthirius multifiliis* by use of murine monoclonal antibodies. Infection and Immunity, 64: 4085-4091.
17. Burkart, M.A., T.G. Clark and H.W. Dickeron, 1990. Immunization of channel catfish, *Ictalurus punctatus* Rafinesque against *Ichthyophthirius multifiliis* (Fouquet): killed versus live vaccine. J. Fish Diseases, 13: 401-410.
18. Buchmann, K., T. Lindenstrøm and J. Sigh, 1999. Partial cross-protection against *Ichthyophthirius multifiliis* in *Gyrodactylus derjavini* immunized rainbow trout. J. Helminthology, 73: 189-195.
19. McCallum, H.I., 1986. Acquired resistance of Black mollies *Poecilia latipinna* to infection by *Ichthyophthirius multifiliis*. Parasitology, 93: 251-261.
20. Noe, J.G. and H.W. Dickerson, 1995. Sustained growth of *Ichthyophthirius multifiliis* at low temperature in the laboratory. J. Parasitol., 81(6): 1022-1024.
21. Derwa, H.I.M., 2004. Studies on the role of some insecticides on the prevalence of protozoal diseases in some freshwater fishes. Thesis of Ph.D. Faculty. of Veterinary. Medicine. Suez Canal University.
22. Osman, H.A.M., 2005. Studies on Monogeuiasis among fish. Thesis of Ph.D. Faculty. of Veterinary. Medicine. Suez Canal University.
23. Xu, D.H., P.H. Klesius and R.A. Shelby, 2004. Immune responses and host protection of channel catfish, *Ictalurus punctatus* (Rafinesque) against *Ichthyophthirius multifiliis* after immunization with live theronts and sonicated trophonts. J. Fish Dis., 27: 135-141.
24. Hai, X., P.H. Klesius and C.A. Shoemaker, 2008. Protective immunity of Nile tilapia against *Ichthyophthirius multifiliis* post – immunization with live theronts and sonicated trophonts. Fish and Shellfish immunology, 2008.
25. Newman, S.G. and J. Majnarich, 1982. Direct immersion vaccination of Juvenil rainbow trout and Juvenil Coho Salmon with *Yersinia ruckeri* bacterin. J. Fish Diseases,5: 339-341.

26. Khalifa, K.A., K.M. Al-Khayat and F.H. Al-Rijab, 1983. Icthyophthiriasis in farmed fishes in Iraq. J. Wildlife Diseases, 19(2): 129-132.
27. Lipton, A.P. and M. Lakshmanan, 1986. *Ichthyophthirius multifiliis* Fouquet (Protozoa: Ciliata) infection in *Channa striatus* (Bloch): experimental induction with notes on treatment. Current Science, India, 54(21): 1123-1126.
28. Ewing, M.S. and K.M. Kocan, 1992. Invasion and development strategies of *Ichthyophthirius multifiliis*, a parasitic ciliate of fish. Parasitology Today, 8(6): 204-208.
29. Woo, P.T.K., 1995. Fish Diseases and Disorders. Volume 1 Protozoan and Metazoan Infections. CAB international. pp: 187.
30. Durborow, M.R., J.A. Mitchell and M.D. Crosby, 1998. Ich (White Spot Disease). SRAC publication No. 476.
31. Lom, J. and I. Dykova, 1992. Protozoan parasites of fishes. Developments in Aquaculture and Fisheries Science 26, Elsevier, Amsterdam.
32. Hoffman, G., 1999. Parasites of North American Freshwater Fishes. Comstock Publishing associates, Ithaca and London.
33. Dickerson, H.W., J. Brown, D.L. Dawe and J.B. Gratzek, 1984. *Tetrahymena pyriformis* as a protective antigen against *Ichthyophthirius multifiliis* infection: comparison between isolates and ciliary preparations. J. Fish Biol., 24: 523-528.
34. Ewing, M.S., K.M. Kocan and S.A. Ewing, 1985. *Ichthyophthirius multifiliis* (Ciliophora) invasion of gill epithelium. J. Protozool, 32: 305-310.
35. Clayton, G.M. and D.J. Price, 1988. *Ichthyophthirius multifiliis:* standardization of the infection-response model in *Ameca splendens* (Miller & Fitzsimons). Journal of Fish Diseases, 11(5): 371-377.
36. Ventura, M.T. and I. Paperna, 1985. Histopathology of *Ichthyophthirius multifiliis* infection in fishes. J. Fish Biology, 27: 185-203.
37. Cross, M.L., 1994. Localized cellular responses to *Ichthyophthirius multifiliis*: Protection or pathogenesis? Parasitol. Today, 10: 364-368.

(Received: 26/11/2008; Accepted: 13/-1/2009)