Global Veterinaria 3 (4): 329-334, 2009
ISSN 1992-6197

Protection of Goldfish (*Carassius auratus)* Against *Ichthyophthirius multifiliis* by Immunization with Live Theronts, Trophonts and Sonicated Trophonts

[1]H.A.M. Osman, [2]Maather M. Monier, [3]Omaima A. Abd El Ghany,
[1]Taghreed B. Ibrahim and [2]Mona M. Ismail

[1]Department of Hydrobiology, National Research Center, Giza, Egypt
[2]Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Suez Canal University, Egypt
[3]Department of Fish Diseases, Animal Health Research Institute, Egypt

Abstract: Vaccination of goldfish *Carassius auratus* against *Ichthyophthirius multifiliis* (Ich) was performed with live theronts,trophonts and sonicated trophonts. Immunizotion was carried out by both, bath immersion and intrapertoneal (IP) injection. Host protection was assessed by detection of humoral immune response, Coetaneous and serum anti – Ich antibody levels were measured 14 and 21 days post immunization by immobilization assay to determine the highest dilution of antibody in skin or serum which make immobilization of all live theronts. The level of Ich infection and survival of goldfish were determined after theronts challenge. Coetaneous and serum anti – Ich antibodies were significantly higher ($p<0.05$) in fish immunized with live theronts by immersion or IP injection or with trophonts or sonicated trophonts administrated by IP injection, than in fish immunized with trophonts or sonicated trophonts by immersion or non immunized controls. There was a positive correlation between higher levels of anti – Ich antibodies and host survival in the immunized fish.

Key words: *Carassius auratus* · Ich · Theronts · Trophonts · Sonicated · Antibody titre · Vaccination

INTRODUCTION

Goldfish are one of the most popular pets in the world. They are, undoubtedly, characterized by brilliant metallic gold and reddish coloration. White spots is a very common goldfish disease and its scientific name is ichthyophthiriasis caused by an obligate parasite called *Ichthyophthirius multifiliis*. usually occurs due to stressful situations like poor water conditions or a sudden chill. The signs of goldfish Ich were rapid breathing or scratching on the gravel in the aquarium [1]. The white spots, goldfish disease, are the parasites which are feeding on the goldfish's body fluid. White spot disease or Ich can spread quite rapidly on the goldfish if left untreated [1].

It affects cultured and aquarium fishes causing large losses in fish cultures. The ciliate *Ichthyophthirius multifiliis* parasitizes the skin of freshwater teleosts and is considered to be one of the most pathogenic fish protozoans [2, 3].

Fish develop protective immunity against the parasite following immunization with Ich antigens [1,3-8]. Serum and mucus collected from immune fish contained antibodies against the parasite and caused immobilization of theronts and trophonts in vitro [8- 11]. Several studies have shown that coetaneous antibodies in immune fish are involved in protection against pathogens because serum antibody levels do not correlate with acquired protection [12-15]. In an experiment on immunosupression of fish carps, Houghton and Matthews [16] found that the immune carps injected with exogenous corticosteroids were not completely protected against Ich although these fish had been previously infected by the parasite and still had levels of circulating antibodies. Data about the relationship between vaccination, coetaneous immunity and host protection against Ich in fish [6,8] and goldfish (*Carassius auratus*) so the aim of this study is to vaccinate (*Carassius auratus*) with live theronts,trophonts and sonicated trophonts and evaluate humeral immune responses with special references to monitor the coetaneous immune response.

Corresponding Author: Hussein A.M. Osman, Department of Hydrobiology, National Research Center, Postal code: 12622, Dokki, Giza, Egypt

MATERIAL AND METHODS

Fish: Goldfish with a mean length 10.5±1.2 cm and weight 17.5±5 g with no previous history of infection with Ich were used in the current study. Fish were obtained from private ornamental fish breeders at Giza governorate and maintained in tanks supplied with dechlorinated tap water at 24-27°C. Quality of water was maintained by filtration and aeration.

Parasites: Trophonts of Ichthyophthirius was isolated from natural heavy infected goldfish (*Carassius auratus*) obtained from a shop for pet fishes. Isolation of trophonts from infected fish was by immersion in sterile water for 15 minutes then gentle scraping of skin, the parasite was maintained in the laboratory by serial transmission on gold fish held in 50 L glass aquaria equipped with filters. Part of isolated trophonts was placed in Petri dishes with fresh dechlorinated water to remove contaminating mucus and skin debris,. The trophonts were incubated for 18h at 24°C to get theronts. Theronts were harvested by pouring through a sieve with a pore size of 45µm for IP injection,. Theronts were concentrated by centrifugation at 1000 g for 5 min for challenge.

Preparation of Trophont & Theront Antigen

A. Trophonts Collection: Natural heavily infected goldfish with maturing trophonts (white spots) were killed then rinsed with sterile water and kept in a 1L beaker with 100 ml dechlorinated water. Collection was by scraping the skin of infected goldfish, trophonts were collected in a beaker filled with freshwater. The process was repeated. The collected trophonts were pooled and fixed in 4% formalin. The fixed trophonts were counted with help of Sedgewick Rafter cell then washed with phosphate buffered saline (PH 7).Part of trophonts was sonicated for one hour on grinder in PBS. The sonicated trophonts solution was adjusted in volume with PBS to approximately 0.1 ml per fish (20 trophonts per gram fish) for injection according to Xu *et al*. [8].

B. Live Theronts Collection: Part of isolated trophonts were was placed in Petri dishes with fresh dechlorinated water to remove contaminating mucus and skin debris,. The trophonts were incubated for 18h at 24°C. Theronts were harvested by pouring through a sieve with a pore size of 45µm for IP injection,. Theronts were concentrated by centrifugation at 1000 g for 5 min (for challenge with theronts)

Experimental Design and Immunization Procedure: The experiment consisted of 14 tanks each containing 10 fish these fish were divided into 7 replicated groups (two tanks per group) and immunized as follows:

First group was treated with live theronts by immersion at a dose of 20000 theronts per fish,. Second group was treated with live theronts by IP injection at a dose of 20000 theronts per fish. Third group was treated with sonicated (grinded) trophonts by IP injection with 0.1 ml antigen/fish. Fourth group was treated with sonicated trophonts by immersion at a dose of 2000 trophonts per fish., Fivth group was treated with trophonts by IP injection at a dose of 20 trophonts per gram fish. Sixth group was supplied with trophonts by immersion at a dose of 2000 trophonts per fish and seventh group was non immunized infected control one. Fish in immunized groups 2nd, 3rd and 5th groups were anaesthized with clove oil (50 ml/L) weighted and injected IP with antigen 0.1 ml per fish. While 1st, 4th, 6th and 7th groups were placed in tanks with water volume adjusted to 10 L in each tank live theronts and trophonts in 500 ml water, sonicated trophonts in 50 ml tank water. Fish were exposed to the parasite (trophonts or theronts) for 1 hour then transferred to another tanks free from parasite.

Challenge and Determination of Infection Level: After water was adjusted to 10 L in each of 14 tanks (10 fish per tank) at 21 days post – immunization, theronts were added to each tank at a dose of 15000 theronts per fish. Fish were exposed to theronts for 5 hours,. Infection level for each fish was determined when fish showed visible trophonts 3 days after challenge. Fish were put in 2 L beaker with 1000 ml water containing clove oil (50 ml / L). The number of trophonts on the fish skin was counted by magnified lens and Ich infection was determined by scoring 0, < 30 (+), 30 – 60 (++) and > 60 (+++) trophonts per fish. Fish were then returned to their tanks and clinical signs and mortality of fish in each tank was recorded daily for 21 days after theront challenge.

Coetaneous Antibodies and Serum Sampling: 3 fish from each tank (6 fish per immunized group were sampled) 14 and 21 days after immunization the fish were anaesthetized with clove oil (50 ml/L). Blood was collected from caudal vein to get serum samples. The fish was washed with sterile water according to Xu *et al*.[17]. Skin was collected from the lateral body wall with sterile instruments. Approximately 200 mg of skin was collected from each fish, weighted and cut into 5x5 mm pieces. The excised skin was washed with Hank's balanced salt solution (Sigma) and Medium 199 (Sigma) and inoculated

into a well of 24 well culture plate. An amount of culture medium equal to the skin weight was added to each well. The culture medium was made by supplementing Medium 199 with 5% heat inactivated foetal bovine serum (Sigma) 100 IU of penicillin ml^{-1} and 100 µg streptomycin ml^{-1}. The culture fluid was collected 24h after skin incubation and centrifugation at 228 g for 10 min. and the supernatant was collected [17].Both serum and culture fluid were heat inactivated at 56°C for 30 min for decomplementation. and stored at -20°C tell use. inactivation to be sure that the culture fluid was the effective. The immobilization substance in the culture fluid was not a heat – labile complement because the culture fluids were heat inactivated before use [17].

Anti Ich Antibodies in Skin and Serum: Coetaneous and serum anti Ich antibodies were determined by theronts immobilization assay. Immobilization assay was conducted by placing definite live theronts in a dilution series of skin culture fluid or serum in 96 well flat bottomed microtitre plate (Corning coastar) as described by [10, 11, 17] 50 % diluted PBS was used as diluent. Immobilization titer was the highest dilution in which all theronts were immobilized. The relative percent survival (RPS) for each group was calculated according to Newman and Majnarich [18].

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

Statistical Analysis: Coetaneous and serum antibody titers in each immunized group were analyzed with Duncan's multiple range test. Coetaneous antibody, serum antibody and fish survival were evaluated by spearman rank correlation probabilities of 0.05 or less were considered statistically significant.

RESULTS

Clinical Signs of Infected Gold Fish with Ich: The clinical signs and behavioral change of naturally diseased and experimentally infected goldfish with Ich revealed presence of large number of white spots all over the body surface and fins Fig. 1(a), there was increased mucus production from all the body. Infected fish swim rapidly to rube their bodies against sides of aquarium as the disease progress, the fish gasp for oxygen and became lethargic and stoped feeding. Examination of wet mounts of dislodged parasites from the skin and gills, revealed oval to round shape large ciliated parasites (trophonts) with horseshoe – shaped macronucleus Fig. 1 (b&c).

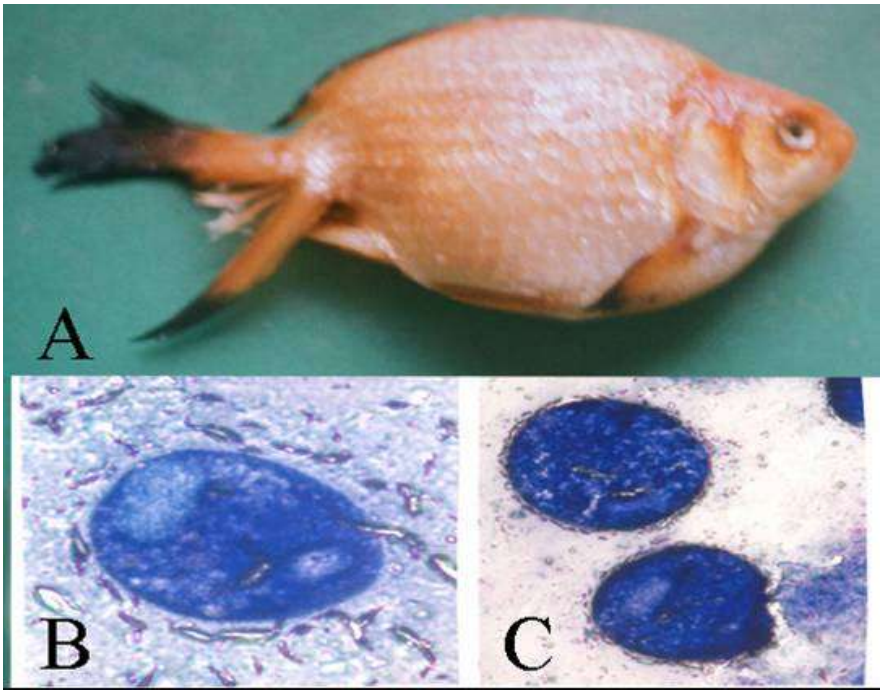

Fig. 1: (A)Showing goldfish (*Carassius auratus*)infected with large numbers of white spots on the head, dorsal side and peduncle (+++) (B&C) showing dislodged Ichthyophthirius multifiliis trophonts in preparation stained with Gaiemsa stain (X 200)

Table 1: Immobilization titres in skin and serum of *Carassius auratus* 14 and 21 days post-immunization against Ich

Treatment				
Site of antibodies	Skin		Serum	
Time / Day	14 D	21 D	14 D	21 D
* Theront immersion	53 a**	72 a	479 a	633 a
* Theront IP	61 a	65 a	469 a	654 a
* Sonicated trophont IP	37 a	42 a	182 a	112 a
* Sonicated traphont immersion	7 c	9 c	15 c	41 c
* Trophont IP	16 b	18 b	31 b	28 b
* Trophont immersion	2 c	4 c	3 c	2 c
* Non – immunized (control)	3 c	2 c	5 c	3 c

** Each value is the mean of four samples within a column, mean values followed by the same lower case letter are not significantly different (p> 0.05) with Dumcan's multiple range test.

Table 2: Infection levels in immunized goldfish after challenge with live theronts (15000 theronts per fish)

Immunized group	No. of fish	non infected	<30 +	30-60 ++	> 60 +++
* Theront immersion	10	10	0	0	0
* Theront IP	10	10	0	0	0
* Sanicated trophont IP	10	5	3	2	0
* Sanicated traphont immersion	10	1	4	3	2
* Trophont IP	10	2	5	1	2
* Trophont immersion	10	0	7	2	1
* Non – immunized (control)	10	0	2	5	3

Table 3: Mortality, Relative Percent Survival (RPS) of immunized goldfish following challenge by exposure to theronts of Ich at a dose of 15000 theronts per fish for 5 hours and observed for 21 days

Immunized groups	No. of fish	No. of died fish (mortality)	Survival%	RPS
* Theront immersion	10	0	100	1
* Theront I/P	10	0	100	1
* Sonicated trophont I/P	10	4	60	0.6
* Sonicated traphont immersion	10	10	0	0
* Trophont I/P	10	7	30	0.3
* Trophont immersion	10	10	0	0
* Non – immunized (control)	10	10	0	0

Anti-Ich Antibodies: Coetaneous or serum anti – Ich antibodies were not detected in fish sampled prior to immunization [goldfish with no history of infection with Ich]. Fish immunized with live theronts by immersion or IP injection with sonicated trophonts or IP injection with trophonts had increased coetaneous and serum anti -Ich antibodies at 14 and 21 days post immunization as measured by immobilization assay table (1). Fish immunized with sonicated trophonts by immersion or trophonts by immersion or non immunized showed low levels or no coetaneous and serum anti-Ich.

Infection Level: After challenge with live theronts 15000 per each fish, no trophont was observed on fish immunized with live theronts by either immersion or IP injection table (2). Fish immunized with trophonts or sonicated trophonts by IP injection or immersion showed visible trophonts also non immunized control showed trophonts with different degrees of infection from light (+) to heavy (+++)

Fish immunized with live theronts by immersion or IP injection all were survived. 60% and 30% of fish immunized by IP injection with sonicated trophonts or trophonts were survived respectively. Fish administrated sonicated trophonts or trophonts by immersion or non – immunized control were not protected 100% mortality, Table (3)

There was a positive correlation between coetaneous anti-Ich antibodies and host survival in immunized fish.

DISCUSSION

The clinical signs recorded in infected fish (++) or (+++) degree of infection in goldfish revealed the same clinical signs recorded by [1, 19, 8, 3]. Results of wet mounts of dislodged parasites (trophonts) under microscope revealed oval to round shop ciliated parasites with its characteristic horseshoe – shaped macronucleus, these findings were coincide with that recorded by many authors [3,19-23]. Present study form a trial to vaccinate gold fish *Carassius auratus* to protect it from infection with Ich, which induce complete destruction to its colored skin losing its economical value accompanied with great deaths to the infected fish itself [4] vaccinated channel catfish, resulted in complete protection when immunized with live theronts at 20000 theronts per fish with IP injection these results nearly agree with present study where vaccination of goldfish with live theronts IP or immersion induce high protection levels of vaccinated goldfish when challenged with 15000 theronts per fish [7] immunized channel catfish on day 1 with 8000 live theronts per fish and poster dose on day 35 with 10000 live theronts per fish by I/P injection, the immunized fish challenged by exposure to 15000 theronts per fish 84 days after the first injection and 59.2% survived. The immunized fish showed a high serum anti – Ich antibody titre from weeks 2 to 11 post immunization, low level of anti – Ich antibodies was detected in mucus of Immunized fish, these results confirmed by the results of present study where immunized goldfish with live theronts by immersion or injection developed a protective humeral immune response against the live theronts challenge.

These nearly agree with the results of Osman *et al.*[4] and Dalgaard *et al*.[6]. Xu, *et al*.[8] reported that the channel catfish immunized and completely protected by injection live theronts and showed high levels of anti – Ich antibodies in serum and skin. Osman *et al.*[[4] also reported that immunized channel catfish with sonicated trophonts at 200 trophonts per fish resulted in 49% survival. Dalgaard *et al*.[6] found that rainbow trout immunized with sonicated trophonts by I/P injection at rates of 10 – 20 trophonts per gram of fish showed lower infection levels than fish immunized with sonicated trophonts by immersion or non – immunized control. These results go hand by hand with the results of the present study. Xu, *et al*.[8,17] found that channel catfish immunized with sonicated trophonts by immersion or non – immunized control showed low or no cutaneous anti – Ich antibodies has a great role in protection of channel catfish against Ich infection specific anti – Ich antibody level appear to be a better indicator of Ich immunity against Ich infection. Present study a trial of immunization with trophonts as it is without sonication by both injection or immersion. Goldfish immunized by such methods of immunization showed incomplete protective levels of both coetaneous or serum anti – Ich antibodies. this may be due to that the sonication of trophonts make it more resemble to the parasite.

In conclusion goldfish immunized with live theronts by immersion or injection acquired high levels of immunity and protection against infection with Ich. Goldfish immunized with sonicated trophonts and trophonts without sonication by injection acquired low levels of protective immunity goldfish immunized with sonicated trophonts or trophonts without sonication by immersion acquired no immunity and revealed mortality rate similar to non immunized control goldfish *Carassius auratus*.

REFERENCES

1. 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.
2. Dickerson, H. and T. Clark, 1998. Ichthyophthirius multifilus a model of cutaneous infection and immunity in fishes. Immunological Reviews, 166: 377-384.
3. Osman, H.A.M., F.B. Laya, A.E. Noor EL Deen and O.K. Abd EL-Hady, 2009. Investigations on white spots disease (*Ichthyophthriasis*) in Catfish(*Clarias gariepinus*) with special reference to the immune response. Global Veterinaria, 3: 113-119.
4. 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. Journal of Fish Diseases, 13: 401-410.
5. 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.
6. Dalgaard, M., A. Li and K. Buchmann, 2002. Immunisation of rainbow trout fry with *Ichthyophthirius multifiliis* sonicate: protection of host and immunological changes. Bulletin of the European association of fish pathologists, 22: 287-297.
7. Wang, X. and H.W. Dickerson, 2002. Surface immobilization antigen of the ciliate Ichthyophthirius multifiliis elicits protective immunity in channel catfish (Ictalurus punctatus). Clinical and Diagnostic Laboratory Immunology, pp: 176-181.
8. 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. Journal of Fish Diseases, 27: 135-141.
9. 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.
10. 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.
11. 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. Journal of Fish Diseases, 24: 49-51.
12. Lobb, C.J. and L.W. Clem, 1981. The metabolic relationships of the immunoglobulins in fish serum, cutaneous mucous and bile. Journal of Immunology, 127: 1525-1529.
13. Lobb, C.J., 1987. Secretory immunity induced in catfish, Ictalurus punctatus, following bath immunization. Developmental and comparative Immunology and Immunopathology, 11: 727-738.

14. Rombout, H.W.M., N. Taverne, M. Van de Kamp and A.J. Taverne Thiele, 1993. Differences in mucous and serum immunoglobulin of Carp (Cyprinus carpio L.). Developmental and Comparative Immunology, 17: 309-317.
15. Cain, K.D., D.R. Johns and R.L. Raison 2000. Characterization of mucosal and systemic immune responses in rainbow trout (Oncorhynchus mykiss) using surface plasmo resonance. Fish and shellfish Immunology, 10: 651-666.
16. 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. Journal of Fish. Diseases, 13: 269-280.
17. Xu, D., P.H. Klesius, C.A. Shoemaker and J.J. Evans, 2002. The early development of *Ichthyophthirius multifiliis* in Channel Catfish inVitro. Journal of Aquatic Animal Health, 12: 290-296.
18. Newman, S.G. and J. Majnarich, 1982. Direct immersion vaccination of Juvenil rainbow trout and Juvenil Coho Salmon with Yersinia ruckeri bacterin. Journal of Fish Diseases, 5: 339-341.
19. 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.
20. Lom, J. and I. Dykova, 1992. Protozoan parasites of fishes. Developments in Aquaculture and Fisheries Science 26, Elsevier, Amsterdam.
21. Woo, P.T.K., 1995. Fish Diseases and Disorders. Volume 1 Protozoan and Metazoan Infections. CAB international. pp: 187.
22. Durborow, M.R., J.A. Mitchell and M.D. Crosby, 1998. Ich (White Spot Disease). SRAC publication No. 476.
23. Hoffman, G., 1999. Parasites of North American Freshwater Fishes. Comstock Publishing associates, Ithaca and London.

(Received: 30/03/2009; Accepted: 20/05/2009)