



Short Review

Does Rohu (*Labeo rohita*) (F. Hamilton, 1822) have different forms of Immunoglobulins?

P. P. Suresh Babu^{1*}, K. M. Shankar², B. R. Honnananda³ and P. B. Abhiman²

¹Calicut Research Centre of ICAR-Central Marine Fisheries Research Institute, West Hill, Calicut - 673 005

²Fish Pathology and Biotechnology Laboratory, Department of Aquaculture, College of Fisheries, Mangalore - 575 002, India

³College of Fisheries, Chhattisgarh Kamdhenu Vishwavidyalaya, Kawardha (Kabirdham) - 491 995, India

Abstract

Characterisation of immunoglobulin (Ig) is an essential prerequisite for development of anti-fish Ig antibody. There are number of reports describing the characteristics of rohu Ig which state the existence of different forms of antibodies in rohu. The present review is an attempt to compile these reports for analyzing the probable reason for the existence of such forms and the possible future research requirements for confirming the same.

Key words: Rohu, immunoglobulin, heavy chain, light chain, Ig forms

Introduction

Rohu, *Labeo rohita* (F. Hamilton, 1822) is the most widely cultivated Indian major carp that contributes around 0.5 million tonnes to the aquaculture production of India. It occupies 13th position among the cultured species of aquatic organisms in the world (FAO, 2005). Rohu is widely cultured in the Indian subcontinent especially, India and Bangladesh. Being the highest contributor to the aquaculture production in India during the last decade it provides about 35% of the total carp production (FAO, 2001).

Formation of a low molecular weight antibody class from the Immunoglobulin (Ig) M that occurs during

the course of an immune response in vertebrates is not manifested in fishes (Isbell & Pauley, 1983). So only one predominant antibody type was thought to be present in fishes which is a high molecular weight Ig often referred to as IgM or IgM like because these molecules have a heavy chain isotype similar to the mammalian μ chain (Wilson & Warr, 1992; Van Muiswinkel, 1995). Characteristics of IgM of a number of commercially important fishes such as Channel catfish (Phillips & Ourth, 1986), Asian catfish (Swain et al., 2004), Sea bass (Palenzuela et al., 1996), Common carp (Zhong et al., 1999), Grouper (Cheng et al., 2006), Snake head (Sood et al., 2011), Magur (Sood et al., 2012) etc. has been reported earlier.

Purification and characterization of Ig is an important aspect in development of monoclonal antibodies for fish Ig which can be employed for several purposes such as to quantify the total Ig in sera at different conditions like healthy, infected, and vaccinated fishes (Wagner et al., 2001; Bobadilla et al., 2004); to detect change in total Ig levels at different seasons, environmental conditions and life stages (Cuesta et al., 2004; Pettersen et al., 2005); to compare the serum antibodies of fishes from different taxa (Al-Harbi et al., 2000; Morrison et al., 2002); to study the heterogeneity in structure of antibodies (Sanchez et al., 1995); to study the ontogeny of antibody production (Breuil et al., 1997); to study the major lymphoid tissues (Pettersen et al., 2000; Jang et al., 2004) and to study the structural changes in fish Ig under different physical, chemical and biological conditions (Magnadottir et al., 1996). In addition the similarities and variations in structure of different Igs were

Received 23 December 2015; Revised 05 July 2017; Accepted 30 August 2017

*E-mail: sbabu78@yahoo.com

demonstrated using fish Ig MAbs (Adkison et al., 1996; Pettersen et al., 1995; Jang et al., 2004; Sanchez et al., 1995; Magnadottir et al., 1996; Sanchez & Dominguez, 1991).

Several reports on the characterisation of Ig of rohu and the development of MAbs and polyclonal antibodies (PAbs) for the Ig are available now. The present paper is an effort to compile all the scientific literature to understand whether rohu is possessing different forms of serum immunoglobulin or not.

Characteristics of rohu Ig

Immunoglobulin of rohu has been raised, purified and characterized by several authors using different techniques and the results are enlisted in Table 1. The actual tetrameric form of Ig was first reported by Suresh Babu et al. (2008) where in a native Ig having a molecular weight (N) of 850 KD with a heavy chain (H. C) of 85 KD and light chain (L.C) of 23 KD has been described. Later, similar observations were reported by Rathore et al. (2008) (N=880 KD, H.C =77.4 KD, L.C = 26.4) and Bag et al. (2009) (H.C =88 KD, L.C = 26 KD). The slight variations obtained in values in all these studies are probably due to the glycosylation of the Ig, which causes anomalous migration on SDS-PAGE gel (Wilson et al., 1985). Similar tetrameric form of Ig was reported from other fishes such as turbot (835 KD, Estevez et al., 1993), African catfish (840 KD, Rathore et al., 2006), Cod (851 KD, Pilstrom &

Petersson, 1991), Atlantic salmon (800 KD, Havarstein et al., 1988), sea bass (883 KD, Bourmaud et al., 1995) and tilapia (900 KD, Rajavarthini et al., 2000) etc.

Different forms of rohu Ig

Other than the tetrameric structure, other forms of Ig such as monomers and dimers also were reported from rohu. Earlier studies on *L. rohita* by Mohanty, (2001) reported different populations of immunoglobulin molecules in the purified sera in non-reducing SDS PAGE. Swain et al. (2006) demonstrated a single 210 KD polypeptide of *L. rohita* Ig like molecule from immunised brood fish serum, egg and larval extracts in non-reducing SDS-PAGE. Rathore et al. (2008) also reported two other peaks in the gel filtration chromatogram with molecular weight 443 KD and 240 KD presumed as the monomer and dimer respectively. Variations in Ig structure have been reported from other fishes also. In Southern blue fin tuna two different forms have been reported one with a molecular weight of 778 KD and the other with 160-180 KD (Watts et al., 2001). Based on the estimated MW, the relative carbohydrate content and the reactivity with anti-halibut IgM anti-sera, Grove et al. (2006) has reported different forms of Ig in Atlantic halibut in addition to the tetrameric form. Deviations in Ig structures were reported from other fishes such as catfishes (Lobb & Clem, 1983) and European Perch (Whittington, 1993) also.

Table 1. Details of immunoglobulin of rohu reported by different authors

	Reference	Molecular weight (KD) of Ig	Antigen used	Purification technique	Molecular weight
1	Mohanty et al. (1998)	Different populations of I g molecules	<i>Edwardsiella tarda</i>	Gel filtration and ion-exchange chromatography	SDS PAGE
2	Swaine et al. (2006)	210	-	Ammonium sulphate precipitation	SDS PAGE
3	Suresh Babu et al. (2008)	850	Bovine serum albumin	BSA-CL agarose column chromatography	SDS PAGE
4	Bag et al. (2009)	908	Bovine serum albumin	BSA-CL agarose column chromatography	SDS-PAGE
5	Rathore et al. (2008)	880 443 240	Bovine serum albumin	BSA-CL agarose column chromatography	Gel filtration chromatography, Gradient gel electrophoresis,

Possible reasons for variation in Ig characteristics in rohu

One of the possible reasons for reporting such forms is the degradation of native Ig molecules. Degradation of Ig can be due to the fluctuation in storage temperature (Acton et al., 1971), enzymatic action (Van Ginkel et al., 1991; Magnadottir et al., 1996) or chemical denaturation (Kaattari et al., 1998; Watts et al., 2001). Up on denaturation with SDS, a portion of the tetrameric teleost IgM has been shown to split into mono, di and tetramers, depending on the species (Lobb & Clem, 1981; Kobayashi et al., 1982; Glynn et al., 1990; Whittington 1993; Pucci et al., 2003; Bromage et al., 2004). But the variations in the molecular weight in the case of rohu cannot be confirmed as because of the degradation of the native Ig because these molecules are purified employing different purification techniques and all these purification methods may not be having the degrading factor.

IgM is the only antibody isotype found universally in gnathostomes (Bengtén et al., 1991; Hordvik et al., 1992; Warr, 1995; Nakao et al., 1998) and until last decade, teleosts were thought to possess only IgM (Savan et al., 2005). However, Clem (1971) reported a truncated form of Ig from *Epinephelus itaira* which is termed as IgY. There have been recent reports regarding different isotypes of fish Ig in teleosts. In teleost three types of Igs have been reported namely, IgM (Bengtén et al., 1991; Hordvik et al., 1992; Warr, 1995; Nakao et al., 1998) IgD (Wilson et al., 1997; Hordvik et al., 1999; Stenvik & Jorgensen, 2000; Hirono et al., 2003) and IgZ or IgT (Danilova et al., 2005; Hansen et al., 2005). IgT was discovered after the genomic analysis of several teleost fishes (Hansen et al., 2005; Danilova et al., 2005; Savan et al., 2005; Buonocore, 2017). This form of Ig was reported as a monomeric Ig in the serum and as a polymeric form in the mucus (Zhang et al., 2010). In rainbow trout the molecular mass of the native IgT was reported as 180 KD with a heavy and light chain of 75 KD and 25 KD respectively (Hansen et al., 2005). The low molecular weight of Ig like structures reported from rohu serum may be an Ig form of any one of these forms. Recently Hordvik (2015) reported three major immunoglobulin (Ig) isotypes such as IgM, IgD and IgT, in *salmonid* fish defined by the heavy chains μ , δ and τ , respectively. Kamil et al. (2013) reported IgM sub-variants in salmonid fish. Kamil et al. (2011) reported two IgM heavy chain isotypes in Atlantic salmon and brown trout.

Recently Dash & Sahoo (2015) reported the presence of IgZ transcripts in rohu through expression studies. Kar et al. (2015) reported the expression of IgM, IgD and IgZ in different tissues of infected rohu. Eventhough these studies indicate the possibilities of the presence of new types of Ig in rohu, the proteomic characterization of the native form of these Igs in rohu is yet to be fulfilled.

Future directions

Since all the reports regarding the existence of low molecular weight Ig in rohu are ambiguous and are preliminary in nature an elaborative study on these aspects is required urgently. A holistic approach by employing both genomic and proteomic analysis for characterizing rohu Ig is the need of the hour. Amino acid sequencing, epitope comparison among the different forms of rohu Ig employing monoclonal antibodies and detailed structural analysis using electron microscopy will give a fair idea about the structural details about these Ig forms. Further studies for evaluating the functional genes involved in the expression of such Ig forms through genome analysis will hopefully solve the issue of the credibility of existence of such Ig forms.

Reference

- Acton, R. T., Weinheimer, P. F., Hall, S. J., Niedermeier, W., Shelton, E. and Bennett, J. C. (1971) Tetrameric immunoglobulins in three orders of bony fishes. *Proc. Natn. Acad. Sci. USA*, 68: 107-111
- Adkison, M. A., Basurco, B. and Hedrick, R. P. (1996) Humoral immunoglobulins of the white sturgeon, *Acipenser transmontanus*: partial characterization of and recognition with monoclonal antibodies. *Dev. Comp. Immunol.* 20: 285-298
- Al-habri, A. H., Trawa, R. and Tune, R. L. (2000) Production and characterization of monoclonal antibodies against tilapia *Oreochromis niloticus* immunoglobulin. *Aquaculture*. 188: 219-227
- Bag, M. R., Makesh, M., Rajendran, K. V. and Mukherjee, S. C. (2009) Characterization of IgM of Indian major carps and their cross-reactivity with anti-fish IgM antibodies. *Fish Shellfish Immunol.* 26: 275-278
- Bengtén, E., Leanderson, T. and Pilstrom, L. (1991) Immunoglobulin heavy chain cDNA from the teleost Atlantic cod (*Gadus morhua* L.) nucleotide sequences of secretory and membrane form show an unusual splicing pattern. *Eur. J. Immunol.* 21: 3027-3033
- Bobadilla, A. S., Redondo, M. J., Macias, M. A., Ferreira, I., Rianza, A. and Alvarez-Pellitero, P. (2004) Development of immunohistochemistry and enzyme-linked

- immunosorbent assays for the detection of circulating antibodies against *Enteromyxum scophthalmi* (Myxozoa) in turbot (*Scophthalmus maximus* L.). Fish Shellfish Immunol. 17: 335-345
- Bourmaud, C. A. F., Romestand, B. and Bouix G. (1995) Isolation and partial characterisation of IgM like Seabass (*Dicentrarchus labrax* L. 1758) immunoglobulins. Aquaculture. 132: 53-58
- Breuil, G., Vassiloglou, B., Pepin, J. F. and Romestand, B., (1997) Ontogeny of IgM-bearing cells and changes in the immunoglobulin M-like protein level (IgM) during larval stages in sea bass (*Dicentrarchus labrax*). Fish Shellfish Immunol. 7: 29-43
- Bromage, E. S., Ye, J. M., Owens, L., Kaattari, I. M. and Kaattari, S. L. (2004) Use of staphylococcal protein A in the analysis of teleost immunoglobulin structural diversity. Dev. Comp. Immunol. 28: 803-814
- Buonocore, F., Stocch, V., Nunez-Ortiz, N., Randelli, E., Gerdol, M., Pallavicini, A., Facchiano, A., Bernini, A., Guerra, L., Scapigliati, G. and Picchietti, S. (2017) Immunoglobulin T from sea bass (*Dicentrarchus labrax* L.): molecular characterization, tissue localization and expression after nodavirus infection. BMC Molecular Biology 18: 8 DOI: 10.1186/s12867-017-0085-0
- Cheng, C., John, J.A.C., Wu, M., Lee, C., Lin, C., Linc and Chang, C. (2006) Characterization of serum immunoglobulin M of grouper and cDNA cloning of its heavy chain. Vet. Immunol. Immunopathol. 109: 255-265
- Clem, L. W. (1971) Phylogeny of immunoglobulin structure and function. IV. Immunoglobulins of the giant grouper, *Epinephelus itaira*. J. Biol. Chem. 10: 9-15
- Cuesta, A., Meseguer, J. and Esteban, M. A. (2004) Total serum immunoglobulin M levels are affected by immunomodulators in seabream (*Sparus auratus* L.). Vet. Immunol Immunopathol. 17: 241-248
- Danilova, N., Bussmann, J., Jekosch, K. and Steiner, L. A. (2005) The immunoglobulin heavy-chain locus in zebra fish: identification and expression of a previously unknown isotype, immunoglobulin Z. Nat Immunol. 6: 229-230
- Dash, P. and Sahoo, P. K. (2015) Ontogeny, tissue distribution and expression analysis of IgZ in rohu, *Labeo rohita* in response to various stimuli. Vet. Immunol Immunopathol. 166: 70-8
- Estevez, J., Leiro, J., Santamarina, A. M., Damainguez, J. and Ubeira, F. M. (1993) Isolation and partial characterisation of Turbot (*Scophthalmus maximus*) immunoglobulins. Com. Biochem. Physiol. 105: 275-281
- FAO (2005) Yearbook on Fishery Statistics. FAO, Rome, Italy
- FAO (2001) Yearbook on Fishery Statistics. FAO, Rome, Italy
- Glynn, P. J. and Pulsoford, A. L. (1990) Isolation and partial characterization of the serum immunoglobulin of the flounder, *Platyichthys flesus*. J. Marine Biol. Ass. United Kingdom. 70: 429-440
- Grove, S., Tryland, M., Press, C. M. and Reitan L. J. (2006) Serum immunoglobulin M in Atlantic halibut (*Hippoglossus hippoglossus*): Characterisation of the molecule and its immune-reactivity. Fish Shell fish Immunol. 20: 97-112
- Hansen, J. D., Landis, E. D. and Philips, R. B. (2005) Discovery of a unique heavy-chain isotype (IgT) in rainbow trout: Implication for distinctive B cell developmental pathway in teleost fish. Proc. Natl. Acad. Sci. 102: 6919-6924
- Haverstein, S. L., Aasjord, P. M., Ness, S. and Endresen, C. (1988) Purification and partial characterization of an IgM-like serum immunoglobulin from Atlantic salmon (*Salmo salar*). Dev. Comp. Immunol. 12: 773-785
- Hirono, I., Nam, B. H., Enomoto, J., Uchno, K. and Aoki, T. (2003) Cloning and characterization of a cDNA encoding Japanese flounder, *Paralichthys olivaceus* IgD. Fish Shellfish Immunol. 15: 63-70
- Hordvik, I. (2015) Immunoglobulin Isotypes in Atlantic Salmon, *Salmo Salar*. Biomolecules. 5: 166-177
- Hordvik, I., Voie, A. M., Glette, J., Male, R. and Endresen, C. (1992) Cloning and sequence analysis of two isotypic IgM heavy chain genes from Atlantic salmon, *Salmo salar* L. Eur. J. Immunol. 22: 2957-2962
- Hordvik, I., Thevarajan, J., Samdal, I., Bastani, N. and Krossoy, B. (1999) Molecular cloning and phylogenetic analysis of the Atlantic salmon immunoglobulin D gene. Scand. J. Immunol. 50: 202-210
- Isbell, G. L. and Pauley, G. B. (1983) Characterization of immunoglobulins from the brown bull head (*Ictalurus nebulosus*) produced against a naturally occurring bacterial pathogen *Aeromonas hydrophila*. Dev. Comp. Immunol. 7: 473-482
- Jang, H. N., Woo, J. K., Cho, Y., Kyong, S. B. and Choi, S. H. (2004) Characterization of monoclonal antibodies against heavy and light chain of flounder *Paralichthys olivaceous* immunoglobulin. J. Biochem. Mol. Biol. 37: 314-319
- Kaattari, S., Evans, D. and Klemer, J. (1998) Varied redox forms of teleost IgM: an alternative to isotypic diversity? Immunol. Rev. 166: 133-142
- Kamil, A., Falk, K., Sharma, A., Raae, A., Berven, F., Koppang, E. O. and Hordvik, I. (2011) A monoclonal antibody distinguishes between two IgM heavy chain isotypes in Atlantic salmon and brown trout: protein characterization, 3D modeling and epitope mapping. Mol. Immunol. 48: 1859-1867

- Kamil, A., Raae, A., Fjelldal, P. G., Koppang, E. O., Fladmark, K. E. and Hordvik, I. (2013) Comparative analysis of IgM sub-variants in salmonid fish and identification of a residue in $\kappa 3$ which is essential for MAb4C10 reactivity. *Fish Shellfish Immunol.* 34: 667-72
- Kar, B., Mohapatra, A., Mohanty, J. and Sahoo, P.K. (2015) Transcriptional changes in three immunoglobulin isotypes of rohu, *Labeo rohita* in response to *Argulus siamensis* infection. *Fish Shellfish Immunol.* 2015 Nov; 47(1): 28-33
- Kobayashi, K., Hara, A., Kazunori, T. and Hirai, H. (1982) Studies on subunit components of immunoglobulin M from a bony fish, the chum salmon (*Oncorhynchus keta*). *Mol. Immunol.* 19: 95-103
- Lobb, C. J. and Clem, L. W. (1983) Distinctive subpopulations of catfish serum antibody and immunoglobulin. *Mol. Immunol.* 20: 811-818
- Lobb, C. J. and Clem, L. W. (1981) Phylogeny of immunoglobulin structure and function X. Humoral immunoglobulin of the sheepshead, *Archosargus probatocephalus*. *Dev. Comp. Immunol.* 5: 271-282
- Magnadottir, B., Kristjansdottir, H. and Gudmundsdottir, S. (1996) Characterisation of monoclonal antibodies to separate epitopes on salmon IgM heavy chain. *Fish Shellfish Immunol.* 6: 185-198
- Mohanty, J. (2001) Purification and characterization of serum immunoglobulins of rohu (*Labeo rohita*). Bhubaneswar, India: PhD thesis, Utkal University
- Mohanty J., Sahoo P. K. and Mukherjee S. C. (1998) Purification of rohu serum immunoglobulins: a preliminary study. *J Aquacult.* 6: 67-72
- Morrison, R. N., Hayball, J. D., Cook, M. T. and Nowak, B. F. (2002) Anti-immunoglobulin binding and activation of snapper (*Pagrus auratus*) leucocytes. *Dev. Comp. Immunol.* 26: 247-255
- Nakao, M., Moritomo, T., Tomana, M., Fujiki, K. and Yano, T. (1998) Isolation of cDNA encoding constant region of the immunoglobulin heavy-chain from common carp (*Cyprinus carpio* L.). *Fish Shellfish Immunol.* 8: 425-434
- Palenzuela, O., Sitja-Bobadilla, A. and Alvarez-Pellitero. (1996) Isolation and partial characterization of serum immunoglobulins from sea bass (*Dicentrarchus labrax* L.) and gilthead sea bream (*Sparus aurata* L.). *Fish Shellfish Immunol.* 6: 81-94
- Pettersen, E. F., Bjorlow, I., Hagland, T. J. and Wergeland, H. I. (2005) Effect of seawater temperature on leucocyte populations in Atlantic salmon post-smolts. *Vet. Immunol. Immunopathol.* 106: 65-76
- Pettersen, E. F., Fyllingen, I., Kavlie, A., Massaeide, N. P., Glette, J., Endresen, C. and Wergeland, H. I. (1995) Monoclonal antibodies reactive with serum IgM and leucocytes from Atlantic Salmon *Salmo salar* L. against sea bass *Dicentrarchus labrax* (L.). *Fish Shellfish Immunol.* 5: 275-287
- Pettersen, E. F., Bjerknes, R. and Wergeland, H. I. (2000) Studies of Atlantic salmon (*Salmo salar* L.) blood, spleen and head kidney leucocytes using specific monoclonal antibodies, immunohistochemistry and flow cytometry. *Fish Shellfish Immunol.* 10: 695-710
- Phillips, J. O. and Ourth, D. D. (1986) Isolation and molecular weight determination of two immunoglobulin heavy chains in the channel catfish, *Ictalurus punctatus*. *Comp. Bioch. Physiol. B.* 85: 49-54
- Pilstrom, L. and Petersson, A., (1991) Isolation and partial characterization of immunoglobulin from cod (*Gadus morhua* L.). *Dev. Comp. Immunol.* 15: 143-152
- Pucci, B., Coscia, M.R. and Oreste, U. (2003) Characterization of serum immunoglobulin M of the Antarctic teleost *Trematomus bernacchii*. *Comp. Bioch. Physiol.* 135: 349-351
- Rajavarthini, P. B., Amukumar, R. I. and Michael, R. D. (2000) Partial characterization of serum immunoglobulin of *Oreochromis mossambicus*. *Indian J. Exp. Biol.* 38: 549-553
- Rathore, G., Swaminathan, T. R., Sood, N., Mishra, B. N. and Kapoor, D. (2006) Affinity purification and partial characterization of IgM like immunoglobulins of African catfish, *Clarias garipinus* (Burchell, 1822). *Indian J. Exp. Biol.* 44: 1081-1021
- Rathore, G., Kumar, G., Sood, N., Kapoor, D. and Lakra, W. S. (2008) Development of monoclonal antibodies to rohu [*Labeo rohita*] immunoglobulins for use in immunoassays. *Fish Shellfish Immunol.* 25: 761-774
- Sanchez, C. and Dominguez, J. (1991) Trout immunoglobulin populations differing in light chains revealed by monoclonal antibodies. *Mol. Immunol.* 28: 1271-1277
- Sanchez, C., Alvarez, A., Castillo, A., Zapata, A., Villena, A. and Dominguez, J., (1995) Two different subpopulations of Ig-bearing cells in lymphoid organs of rainbow trout. *Dev. Comp. Immunol.* 19: 79-86
- Savan, R., Aman, A., Nakao, M., Watanuki, H. and Sakai, M. (2005) Discovery of a novel immunoglobulin heavy chain gene chimera from common carp (*Cyprinus carpio* L.). *Immunogenetics.* 57: 458-463
- Sood, N., Chaudhary, D. K., Rathore, G., Singh, A. and Lakra, W. S. (2011) Monoclonal antibodies to snakehead, *Channa striata* immunoglobulins: Detection and quantification of immunoglobulin-positive cells in blood and lymphoid organs. *Fish Shellfish Immunol.* 30: 569-575
- Sood, N., Chaudhary, D. K., Singh, A. and Rathore, G. (2012) Monoclonal antibody to serum immunoglobulin

- lins of *Clarias batrachus* and its application in immunoassays. *Gene*. 511: 411-419
- Stenvik, J. and Jorgensen, T. O. (2000) Immunoglobulin D (IgD) of Atlantic cod has a unique structure. *Immunogenetics*. 51: 452-461
- Suresh Babu, P. P., Shankar, K. M., Honnananda, B. R., Vijaya Kumara Swamy H. V., Prasanna Shama, K., Suryanarayana, V. V. S. and Dechamma, H.J. (2008) Isolation and characterisation of immunoglobulin of the Indian major carp, rohu [*Labeo rohita* (Ham.)]. *Fish and Shellfish immunology* 24: 779-783
- Swain, P., Dash, S., Bal, J., Routray, P., Sahoo, P. K., Sahoo, S. K., Saurabh, S., Gupta, S. D. and Meher, P. K. (2006) Passive transfer of maternal antibodies and their existence in eggs, larvae and fry of Indian major carp. *Labeo rohita* (Ham.) *Fish Shellfish Immunol.* 20: 519-527
- Swain, P., Mohanty J. and Sahu, A. K. (2004) One step purification and partial characterisation of serum immunoglobulin from Asiatic catfish (*Clarias batrachus* L.). *Fish Shellfish Immunol.* 17: 397-401
- Van Ginkel, F. W., Pascual, D. W. and Clem, L. W. (1991) Proteolytic fragmentation of channel catfish antibodies. *Dev. Comp. Immunol.* 15: 41-51
- Van Muiswinkel, W. B. (1995) The piscine immune system: Innate and acquired immunity. In: *Fish Diseases and Disorders* (Woo, P. T. K., Ed). University press, Cambridge, U.K., pp 729-750
- Wagner, U., Hadge, D., Gudmundsdottir, B. K., Nold, K. and Drossler, K. (2001) Antibody response in salmonids against the 70 kDa serine protease of *Aeromonas salmonicida* studied by a monoclonal antibody-based ELISA. *Vet. Immunol. Immunopathol.* 82: 121-135
- Warr, G. W. (1995) The immunoglobulin genes of fish. *Dev. Comp. Immunol.* 19: 1-12
- Watts, M., Munday, B. L. and Burke, C. M. (2001) Production, characterisation and diagnostic use of antisera to southern bluefin tuna (*Thunnus maccoyii* Casteln) immunoglobulin. *Aquaculture*, 199: 245-257
- Whittington, R. J. (1993) Purification and partial characterisation of serum immunoglobulin of the European Perch (*Perca fluviatilis* L.). *Fish Shellfish Immunol.* 3: 331-343
- Wilson, M. R. and Warr, G. W. (1992) Fish immunoglobulins and the genes that encode them. *Annual Rev. Fish Diseases*. 2: 201-221
- Wilson, M. R., Wang, A. C., Fish, W. W. and Warr, G. W. (1985) Anomalous behaviour of goldfish IgM heavy chain in Sodium dodecylsulfate polyacrylamide gel electrophoresis. *Comp. Biochem. Physiol.* 82: 41-49
- Wilson, M., Bengten, E., Miller, N. W., Clem, L. W., Du Pasquier, L. and Warr, G.W. (1997) A novel chimeric Ig heavy chain from teleost fish shares similarities to IgD. *Proc. Natl. Acad. Sci. USA*, 94: 4593-4597
- Zhang, Y. A., Salinas, I, Li, J., Parra, D, Bjork, S., Xu, Z., LaPatra S. E., Bartholomew, J. and Sunyer, J. O. (2010) IgT, a primitive immunoglobulin class specialized in mucosal immunity. *Nature Immunol.* 11: 827-835
- Zhong, M. C. I., Mor, A. I. and Avtalion, R. R. (1999) One-step procedure for the purification of goldfish (*Carassius auratus*) and carp (*Cyprinus carpio*, L.) serum immunoglobulin by precipitation with 9% polyethylene glycol 6000. *Israeli. J. Aquacult. Bamidgeh.* 1: 3-9