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Mini Review

Ivermectin, its Applications in Aquaculture and Detection Methods

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Abstract

Aquaculture is one of the fastest-growing food-producing sectors worldwide. However, diseases remain a significant challenge for sustainable aquaculture production. Parasitic infestations, particularly in freshwater fishes, are causing economic losses to farmers. Chemotherapeutants are used for direct treatment of these infestations in aquaculture farms. Ivermectin (IVM) is a macrocyclic lactone in the avermectin group, which is used to treat parasitic infections in humans and farmed animals. It is administered orally and is successfully used in treatment of parasitic infestations due to its effect on glutamate-gated chloride channels (GluCls). This review paper discusses Ivermectin of discovery, structure, mechanism of action, applications in aquaculture, and detection methods, focusing on its impact and policy implications in the aquaculture sector.

Keywords: Ivermectin, parasites, glutamate-gated chloride channels, aquaculture, detection methods

Introduction

Ivermectin (IVM) is the most commonly applied drug in human and animal health sectors to treat parasitic infections (Laing, Gillan, & Devaney, 2017). Ticks, mites, and botflies are mainly responsible for substantial economic losses in the livestock industry (Sharma et al., 2021). India loses about 4.45 billion US dollars (20,000 crore rupees) annually due to the adverse effects of certain diseases (Tiwari et al., 2013). IVM is highly effective against ectoparasites and is widely used in the livestock industry to control these parasites. It is also employed for treating parasites in cattle, horses, sheep, and pigs (Omura & Crump, 2004). IVM is used to treat lymphatic filariasis (elephantiasis) and onchocerciasis (river blindness), and it has saved millions of lives in the world's poorest nations through the Mectizan Donation Programme (Laing et al., 2017). IVM is primarily used in the aquaculture sector to manage ectoparasitic copepods, such as salmonid sea lice (*Lepeophtheirus salmonis*) and *Caligus* sp. (Athanassopoulou, Ragias, Roth, Liberis, & Hatzinikolaou, 2002).

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Discovery and synthesis

In 1973, the Kitasato Institute of Japan isolated *Streptomyces avermitilis*, a new actinomycete species, from the NRRL 8165 soil sample collected by Satoshi Omura from Japan. Streptomyces, isolated from the NRRL 8165 soil samples, was tested for its antiparasitic effect in mice against *Nematospiroides dubius* (*Heligmosomoides polygyrus*), and the results revealed its potent antiparasitic activity. The purified active components contain mainly macrocyclic lactones in their chemical structure (Campbell, 1981). These naturally occurring compounds were discovered in 1975 (Omura & Crump, 2004) and

were named avermectins. Avermectins – mainly consist of a mixture of four series, avermectins A1, A2, and B1, B2, with each compound shown as *a* and *b* variants. *In vitro* studies revealed that among all other avermectin series, the avermectin *B* series has more efficacy when tested against gastrointestinal nematode infections in sheep (Campbell, 1981). As a result, the *B* series was the primary target for the development of commercial anthelmintic medications. IVM is a chemically modified derivative of the avermectin B1 series that occurs naturally. It has strong anti-endoparasite and anti-ectoparasite properties for which the term "endectocide" was coined. IVM is the first endectocide that was introduced in animal health sector, and was marketed by Merck Sharp & Dohme Research Laboratories (MSDRL), USA, in 1981 (Omura & Crump, 2004).

IVM has a broad-spectrum activity and is primarily made up of two compound mixtures; ≥ 80% comprising of 22, 23-dihydroavermectin B1a (C48H74O14, B1a) with secondary butyl substitute at the 25-carbon position and ≤ 20% comprising of 22, 23-dihydroavermectin B1b (C47H72O14, B1b) (Fig. 1) (Liebig et al., 2010) which has isopropyl substituent at the 25 position (Campbell, 1981). IVM has a molecular weight of 875.1 g mol^{-1} and is a relatively large molecule with a complex structure. Its log Kow value of 3.2 indicates more hydrophobicity and a strong binding affinity to organic matter (King, 2023)

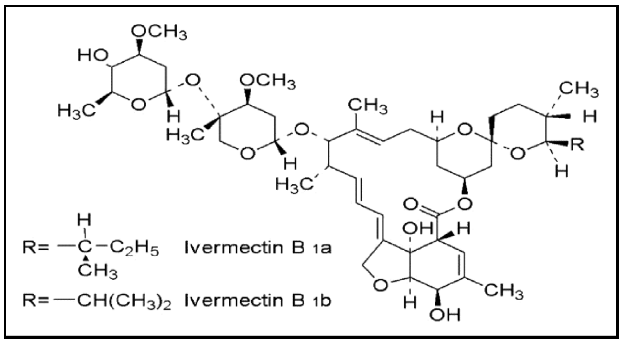

Fig. 1. Picture depicting chemical structure of Ivermectin B1a and Ivermectin B1b (King, 2023)

Mechanism of action

The primary target of Ivermectin (IVM) is the glutamate-gated chloride channels (GluCls), which are commonly found in invertebrate muscle and nerve cells. At nanomolar concentrations, IVM causes irreversible opening of GluCls channels, leading to increased cell membrane permeability and cell hyperpolarization, which results in paralysis and eventual death of the parasites. Additionally, at micromolar concentrations, IVM also has an affinity for other ligand-gated chloride channels (GABA, glycine, and histamine) present in both invertebrates and vertebrates (Gonzalez, Gonzalez, & Ueno, 2012). In mammals, these GluCls are found in the spinal cord and brain. Importantly, IVM cannot cross the blood-brain barrier (BBB) in animals due to a high concentration of P-glycoproteins. Furthermore, it has a low affinity towards other mammalian ligand-gated channels, making it considered very safe for mammals (Turner & Schaeffer, 1989).

IVM administration methods in aquaculture

Avermectins are primarily used in aquaculture to manage ectoparasitic copepods. IVM has broad-spectrum antiparasitic and anthelmintic activities (Alves, Nogueira, Barriga, Dos Santos, Santos, & Tavares Dias, 2019; Oliveira, Brasiliense, Dias, Yoshioka, & Tavares-Dias, 2019) and is efficient in controlling parasitic arthropods and nematodes in aquaculture (Varo et al., 2010). It is available for "off-label" use in aquaculture (i.e., for use in pesticidal applications other than the control of sea lice) (King, 2023). Even though IVM is not licensed for use in aquaculture, there are reports that it is being used occasionally through medicated feed to control sea lice infestation (Horsberg, 2012). IVM is effective even at low dosages and can easily be administered orally, topically, and parenterally (Omura, 2008). In farmed fish, the most widely used oral dosages of IVM range from 0.05 to 0.2 ppm with varying administration feeding rations (e.g., single dose, one or two times a week) and also with no discernible adverse effects on the fish (Palmer, Rodger, Drinan, Dwyer, & Smith, 1987; Johnson, Kent, Whitaker, & Margolis, 1993; Athanassopoulou et al., 2002). The applications of IVM in aquaculture is mostly through diet and bath treatments in different fish species and treatment dosages are shown in Table 1.

Detections methods

Long-term use of IVM may result in drug residues in different animal-derived products such as meat, fish, eggs, and milk, which could be dangerous to public health if its level is greater than Maximum Residue Limits (MRLs) in tissues (European Union Regulation 37/2010/EC). IVM is detected by differ-

Table 1. Ivermectin and its applications in aquaculture

Sl. No.	Fish species	Dosage	Reference
1	*Salmo salar* (Atlantic Salmon)	A single-time feeding with a concentration of 0.20 mg kg^{-1} b.wt. IVM (1% w/v injectable solution Ivomec) was administered.	Palmer et al., 1987
2	*Oncorhynchus mykiss, O. kisutch, O. tshawytscha and Salmo salar*	Fish were given varying dosages of IVM (1% W/V) by oral feeding every other day for 50 days.	Johnson et al., 1993
3	*Dicentrarchus labrax* (sea bass)	IVM given at doses between 0.5 and 3.5 ppm by injection, oral intubation, and through feed.	Athanassopoulou et al., 2002
4	*Sparus aurata* (sea bream)	IVM is administered as a single IP at a single dose of 100 ppb kg^{-1} b.wt.	Katharios, Iliopoulou-Georgudaki, Antimisiaris, Kantzaris, & Pavlidis, 2002
5	*Sparus aurata* (sea bream)	Oral administration of IVM @0.2 $mg.kg^{-1}$ b. wt. fish for ten days	Varo et al., 2010
6	*Salmo salar*	Oral administration of IVM (Eqvalan®) @0.05 and 0.25 ppm kg^{-1} b.wt. once in three days for 30 days (10 treatments).	Ucan-Marin, Ernst, O'Dor, & Sherry, 2012
7	*Oncorhynchus mykiss* (rainbow trout)	Single IP injection at doses of 0.01 & 0.02 $mg.kg^{-1}$ b.wt of IVM.	Sakin, Yonar, Yonar, & Saglam, 2012
8	*Danio rerio* (Zebrafish)	Oral administration of IVM (99% analytical grade) @0.05 & 0.10 mg kg^{-1} b. wt. two times a week for 28 days (16 treatments).	Collymore et al., 2014
9	*Danio rerio* (zebrafish)	Therapeutic bath treatment of IVM at a concentration of 0.25 mg L^{-1} for 4 days and 25 mg L^{-1} for 21 days separately.	Domingues, Oliveira, Soares, & Amorim, 2016
10	*Colossoma macropomum*	Single oral administration IVM (Ivermic Supreme® @ 3.5%) at 4500, 9000, 13500, or 18000 ppm in feed.	Oliveira et al., 2019
11	*Corydoras schwartzi*	Single oral administration IVM transported with polyelectrolytes @ 0.22–170 mg kg^{-1} b.wt.	Madrid et al., 2021
12	*Clarias gariepinus*	Therapeutic bath treatment of IVM at concentrations of 9 to 25mg L^{-1} for four days and 21 days.	Ogueji, Nwani, Mbah, & Nweke, 2019
13	*Clarias gariepinus*	Therapeutic bath treatment of IVM at concentrations of 9 to 25mg L^{-1} for four days.	Ogueji, Nwani, Mbah, Iheanacho, & Nweke, 2020
14	*Prochilodus lineatus*	IVM at a concentration of 0.5 and 1.5 μg L^{-1} for 15 days.	Lozano, Piazza, Babay, Sager, de la Torre, & Nostro, 2021
15	*Cyprinus carpio* (common carp)	Comparative study of therapeutic bath treatment of levamisole, IVM, and fenbendazole with doses of 50 ppm, 0.031, and 25 mg L^{-1}), and IVM @ (mg L^{-1}).	Kolarova, Stara, Zuskova, & Velisek, 2022
16	*Labeo rohita*	Single oral administration IVM@ 200, 300, 500 ppb (μg. $b.wt^{-1}$. of fish).	Hemaprasanth, Kar, Garnayak, Mohanty, Jena, & Sahoo, 2012
17	*Danio rerio* (Zebrafish) *Catla catla* (catla)	Comparative toxicity of bathtreatment of IVM at a concentration of 1, 3, 5, 7, and 9 μg L^{-1} for four days.	Thiripurasundari, Sathya, Uma, Srinivasan, & Rajasekar, 2014

ent analytical equipment, *viz.*, ELISA, High-Performance Liquid Chromatography (HPLC) with fluorescence, and LC-UV and LC fluorescence detection (FLD) (Hernando et al., 2007). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a valuable technique for the analysis of pharmaceuticals due to its high sensitivity and specificity. Different methods employed for detection of IVM are shown in Table 2.

Toxicity studies

The toxicity studies are mainly conducted to determine the standard therapeutic dose of a drug and can be determined by conducting acute and chronic toxicity tests by exposing different concentrations. As IVM is used as a potential antiparasitic and anthelminthic agent in the livestock industry, different toxicity studies were conducted to extend the application of IVM into the aquaculture industry by determining its safe limit. Studies on the toxicity of IVM in in-vivo model in Atlantic salmon and Rainbow trout revealed no toxic effects in fish after receiving a single dose of 0.2 mg kg^{-1} through oral administration after 24 h (Palmer et al., 1987). O'Halloran et al. (1992) observed that Atlantic salmon smolts given a single dose of 0.2 mg IVM kg^{-1} fish showed a slight increase in mortality (0.6%) and lethargy. Additionally, they reported mortality rates (10 - 24%) in Atlantic salmon fed a single dose of 0.4 mg IVM kg^{-1} fish. Similarly, when an oral dose of 0.75 mg IVM kg^{-1} fish was administered to Atlantic salmon, 26% of the fish were found dead (Smith et al., 1993). IVM (Ivermic Supreme®), when administered through oral feeding at a concentration of 3.5% in fingerlings of *Colossoma macropomum*, displayed signs of hypoxia and lethargy after feeding, and died within 10 h (Oliveira et al., 2019). Similarly, *Oncorhynchus mykiss* had acute toxicity values (96 h-LC_{50}) of 3 ppb, bluegill sunfish had LC_{50} values of 4.8 ppb (Halley et al., 1989), and *Salmo salar* had LC_{50} values of 17 ppb (Kilmartin et al., 1996). By comparing the toxicity and pathological effects of IVM on three distinct fish species, namely Coho salmon *(Oncorhynchus kisutch)*, Steelhead trout *(Oncorhynchus mykiss),* and Chinook salmon *(Oncorhynchus tshawytscha*), it was found that Atlantic salmon was more sensitive to IVM toxicity than Chinook and Coho (Johnson et al., 1993).

Behavioral changes

When any xenobiotic is administered to fishes, they may show behavioural responses in accordance with different dosages of drug. As IVM is a potent neurotoxicant the barrier between therapeutic dosage and toxic dosage of IVM, is narrow and fishes

Table 2. Overview of detection methods employed for detection of Ivermectin

S. No.	Fish species	Application	Detection method	Reference
1	*Salvelinus leucomaenis*	Application of IVM @ of 0.3 ppm kg. b.wt^{-1}. through single oral dose and by IP injection.	HPLC-UV	Han, Yang, Wang, & Lu, 2014
2	*Cyprinus carpio haematopterus (brocarded carp)*	Application of IVM (99.5% pure). @ of 0.3 ppm kg.b.wt^{-1}. though single oral dose and by IP injection.	ELISA	Wang, Han, Li, Cao, Du, & Lu, 2019
3	*Fish tissues*	Validation performed at 10 ppb for avermectin compounds such as IVM, Abamectin, Eprinomectin, Doramectin, and Moxidectin in fish tissues.	LC-APCI-MS/MS	Noppe, Verheyden, Vanden Bussche, Wille, & De Brabander, 2019
4	*Sparus auratus* (Seabream)	The Validation performed for Abamectin and Eprinomectin @8 ppb, IVM @16 ppb, and Doramectin and Moxidectin @24 ppb.	LC-ESI (+)-MS/MS	Moschou, Dasenaki, & Thomaidis, 2019
5	Fish and shrimp samples collected in China	Detection of 30 pharmaceuticals (Including IVM) fish samples.	UPLC-MS/MS	Gao et al., 2019

after administration may undergo stress and exhibit behavioural changes such as loss of equilibrium, erratic swimming behaviour, air gulping, hyperpigmentation, mucus secretion etc. In a study conducted by Ucan-Marin et al. (2012), the cumulative mortalities were 5% and 70% for the low and high-dose IVM treatments, respectively, with LD_{50} found to be 0.174 mg kg^{-1} body weight when IVM was added to the diet of Atlantic salmon for 30 days at 10 dose levels ranging from 0.05 to 0.25 mg kg^{-1} body weight. A higher dose of IVM caused the fish to behave erratically, lose their equilibrium, gather at the surface, swim erratically, and sometimes swim in circles with reduced feeding. In another study, IVM was tested for up to four days to determine its acute behavioral changes in juvenile *Clarias gariepinus* at doses of 9 ppm to 25 ppm; in which the juveniles showed extreme sensitivity to IVM with LC_{50} of 15 ppm. As exposure time and concentration increase, behavioral reactions can include erratic movement, loss of balance, color changes in the skin, mucus secretion, cessation of opercular movement, and air-gulping behavior (Ogueji et al., 2019).

Hematological changes

Any drug that enters the body, first diffuses into the blood and exhibits significant changes in haematological parameters such as alteration in total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Volume (MCV) at different dosages which will significantly affect the overall transportation and metabolism system of a living organism. As IVM is more lipophilic, it can easily cross the plasma membrane of blood cell, and exhibit significant changes in haematological parameters in fishes. Lozano et al. (2021) revealed that after 15 days of exposure to varying concentrations of IVM (0.5 ppb, 1.5 ppb, and 0 (control)) in 6 L aquaria, the juveniles of *Prochilodus lineatus* exhibited alterations in their haematological parameters, even at the lowest IVM concentration (0.5 g L^{-1}). During the escape response, GST activity and the maximum swimming speed were significantly reduced at the lowest IVM concentration. In a comparative study, Kolarova et al. (2022) found that therapeutic bath treatment with fenbendazole (25 ppm), levamisole (50 ppm), and IVM (0.031 ppm) in common carp caused changes in the histology and SOD of the carp gills but showed no effect on the haematological and biochemical blood profile. As per Ogueji et al. (2019), hematological responses in juvenile *Clarias gariepinus* at doses of 9 to 25 ppm included white blood cell count displaying a biphasic trend, decreased PCV, Hb, and RBC count. As the concentration of drug increased, the MCHC increased while the MCV and MCH displayed a mixed trend. There was also a notable increase in neutrophils and lymphocytes.

Biochemical and oxidative stress changes

The biochemical and oxidative stress parameters such as changes in levels of blood glucose, vitellogenin, acetylcholinesterase, catalase, and SOD can act as stress markers. Recent studies revealed that IVM can cross blood blood-brain barrier of fish (*Sparus aurata*) because this barrier is poorly developed in fish (Katharios et al., 2004). The fish exposed to 0.25 ppm IVM in the chronic toxicity study showed disturbed swimming behavior, while fish exposed to 25 ppm showed mild spine curvature and darker coloration. The 96-hour LC_{50} value was found to be 73.3 ppm, while there were no effects on VTG or AChE. Fish exposed to 25 ppm showed lower CAT and GST levels. The antioxidant system may also be impacted, as indicated by the decrease in CAT activity (Domingues et al., 2016). IVM caused oxidative stress and changed the fish intercellular enzymes (AST, ALT, and ALP), serum metabolites (protein and glucose), and antioxidant enzyme activities, resulting in a negative impact on fish health. When comparing the treated fish to the control, LPO rose noticeably with the highest value of 33.65 U mg $protein^{-1,}$ indicating a mixed trend of significant increase in SOD. When compared to the control, the concentrations of the antioxidant enzymes GR, CAT, and GPx increased significantly between 24 and 96 h at 9 to 25 μg L^{-1} (Ogueji et al., 2020).

Immunological responses

Most of the chemical compounds have immune suppressive effects after administration in living organisms. White blood cells play an important role in elimination of any foreign particles from the body. IVM causes a decrease in the WBC count and exhibits significant effects on immune system. The effects on specific immunological parameters, antioxidant status, and oxidative stress in the kidney, heart, spleen, liver, and blood were investigated after intraperitoneal injection of IVM at single doses of 0.01 mg and 0.02 mg IVM kg^{-1} in rainbow trout

(Oncorhynchus mykiss). The fish treated with IVM display behavioral abnormalities such as loss of reflexes, erratic swimming, darkening of the body, decreased WBC value, and lethargy (Sakin et al., 2012).

Histopathological changes

When a drug enters the body, fishes may not exhibit any behavioural changes at the lowest concentration. However, the toxicity of drug can be effectively evaluated through the examination of histopathological changes at the inter and intra-cellular levels. As IVM is toxic even at lower dosages, histopathological changes of different tissues show marked changes such as necropsy, apoptosis, pyknotic nuclei and disruption of the cell membrane at tissue level. Histopathological changes were examined in (both 3 g and 35 g) sea bass *Dicentrarchus labrax* IVM at dosages ranging from 0.5 ppm to 3.5 ppm by in-feed, oral intubation, and injection administration. When the substance was fed at 0.5 ppm and 0.7 ppm, no toxicological symptoms were observed. On the other hand, toxicity (> 10%) was noted at dose rates of 0.2 ppm for oral intubation and 0.5 ppm for injection of the compound. The compound was noticeably more toxic when fish were raised at 11°C instead of 20°C. The pathology was mainly limited to the intestinal tissue and gills, as demonstrated by the histopathological examination of the major organs (Athanassopoulou et al., 2002). Similarly, there were mild behavioral changes after receiving IVM twice weekly for up to four weeks at doses of 0.05 mg kg^{-1}.bw and 0.10 mg kg^{-1}.bw. Histology and necropsy investigations show that the internal organs of zebrafish showed no discernible lesions (Collymore et al., 2014).

Gene expression studies

When any xenobiotic enters the fish body, the response at the molecular level can be effectively determined through gene expression studies. A change in the dosage of the drug produced a significant change in the up and down-regulation of genes which indicates stress condition in fishes. As After oral administration of IVM at a recommended dose of 0.2 mg kg^{-1} fish for 10 days, gilthead sea bream juveniles (35 g) were studied using the first proteomic approach to assess the possible hepatotoxicity of IVM (Varo et al., 2010). Under standard culture conditions, the impact of this treatment on the liver protein profile of gilthead sea bream was investigated using Difference Gel Electrophoresis (DIGE). Significant variations in the expression of 36 different protein spots were observed. Among the analyzed protein spots, only six were found to have three positive identifications. These corresponded to hepatic proteins that are involved in energy generation (beta-globin, ATP synthase subunit beta), oxidative stress responses, and lipid metabolism (apoA-I).

Pharmacokinetic studies

Pharmacokinetics studies mainly involve determining the concentration of drugs distributed in different tissues after injesion and metabolism at different intervals. These studies involve processes that involve the entry of drugs into the body until their elimination from the body. Different analytical methods such as HPLC, LCMSMS, UHPLC, etc. are commonly used for the determination of the concentration of drugs in the body. As IVM is used as an antiparasitic drug in the aquaculture industry, studies are conducted for the determination of different pharmacokinetic parameters such as Area under the curve (AUC), Tmax, Cmax, etc. These studies are useful in the determination of the withdrawal period and tissue residual studies of the drug. The intraperitoneal injection (IPT) pharmacokinetics in *Salvelinus leucomaensis* after a single oral dose was studied by Han et al. (2014). Following an oral dose of 0.3 ppm kg^{-1} body weight and an intraperitoneal injection of IVM, samples were collected at various intervals and analyzed using HPLC-UV. The pharmacokinetics of IVM varied depending on the route of administration; intraperitoneal injection resulted in a faster absorption rate than oral administration. Using direct competitive ELISA, the pharmacokinetics of IVM in the serum of cultured sea bream *(Sparus aurata*) following a single intraperitoneal injection of 100 ppb kg^{-1} b.wt. was investigated by Kathario et al. (2002). Two hours after treatment, the highest peak serum concentration was 308.4 ng ml^{-1}. HPLC with DAD for IVM was developed by Carrillo et al. (2023) for screening in feed, water, and soil matrices with good linearity. Avermectins (eprinomectin, emamectin, abamectin, IVM, and doramectin) and Milbemycins (moxidectin) were simultaneously determined in fish tissue using LC-ESI-MS/MS (Moschou et al., 2019). The extraction of avermectin and milbemycin from the fish matrix was carried out using acidified ACN (0.1% HCOOH) and QuEChERS methodology, with relative standard deviations less than 20%, the recov-

eries for all target analytes ranged from 86% to 106%. This method has excellent sensitivity, which was demonstrated by the LODs, which ranged from 0.07 μg kg^{-1} for emamectin to 1.3 μg kg^{-1} for doramamectin.

Withdrawal studies of IVM in aquatic animals

Fish have low absorption rates of Ivermectin, excreting a large portion of the prescribed dose in their faeces. The organs with high lipid content exhibited the highest levels of absorbed Ivermectin. Ivermectin was primarily eliminated in its unaltered form after considerable time in the tissues of the fish that received treatment. Ivermectin has a strong affinity for lipids, soil, and organic matter. It can enter the marine environment by excretion from the bile, fish excrement, and uneaten food pellets. Few studies have been carried out on the withdrawal time required for IVM after treating fish with the drug. Smith et al. (1993) undertook a long-term study of orally administering IVM (0.2 mg kg^{-1}) in Atlantic salmon for controlling sea lice infection and reported that IVM administered through feed reduced 97% sea lice infection with two-week withdrawal periods. This study has been a marker in the residue depletion studies in aquacultured salmon, hybrid tilapia, and channel catfish (Shaikh et al., 2012). They found the presence of parent IVM in body issues in the highest concentrations post dosage study until one day in tilapia and channel catfish, whereas till day 7 in Atlantic salmon. At the end of 21 days, all three compounds were undetected in all three fish species. The European Union (2009) established 49-day withdrawal periods following subcutaneous injection for products containing Ivermectin as the only active ingredient.

Environmental studies

Due to its strong affinity for lipids, soil, and organic matter, IVM can enter the marine environment through different sources. As per risk assessment studies, Ivermectin is likely to build up in the sediments, putting the species living there at risk more than those in the pelagic environment. Although Ivermectin has been demonstrated to be toxic to certain benthic infaunal species in single-species tests, there is no proof that ivermectin treatment of fish has impacted multispecies benthic communities in a field setting. *Cyprinus carpio haematopterus* fish were given a single oral gavage dose of 0.3 mg kg^{-1} body weight of IVM. The medication moved from fish to aquatic plants in the first seven days of the IVM's environmental fate in the aquatic micro-ecological system. It then accumulated in the water and sediment before ending up in the invertebrates (Wang et al., 2019)

Studies in Indian context

Hemaprasanth et al. (2012) demonstrated that heavy *Argulus* infestations in *Labeo rohita* could be successfully cleared with a single oral dose of doramectin at 750 μg kg^{-1} b.wt. and IVM at 500 μg kg^{-1} b.wt. These medications must be administered intramuscularly at 150 μg kg^{-1} b.wt. for adult fishes with severe infestations for rapid and total clearance. IVM and doramectin both offer anti-infection protection for 17 - 18 days after administration. A concentration of 20 mg L^{-1} of doramectin and IVM in water can be used for adult parasites; a lower concentration of the drugs in water at 10 ppm can be used for metanaupliar parasites. Compared to catla fish, zebra fish are more resilient to higher concentrations and extended exposure to IVM, as per Thiripurasundari et al. (2014). More fish deaths and behavioral alterations occurred with extended exposure period and higher IVM concentration. Zebrafish were exposed to 7 μg L^{-1} of IVM for 96 h, during which time they showed signs of neurotoxicity (gliosis and neuron degeneration) and hepatotoxicity (necrosis and acute hepatitis). After a 24-hour exposure, the same concentration (7 μg L^{-1}) of IVM in catla fish caused hepatotoxic signs (vacuolations, hepatic cell degradation) in the liver and neuronal degeneration and necrosis in the brain.

Implication of IVM for food safety

Due to the risk of drug residues in edible tissue of fish, administering IVM can cause safety issues in human. Ivermectin residues were found in fat and liver at the highest concentrations, with high muscle levels surrounding the injection site. To ensure that the amount of Ivermectin found in animal foodstuff is below dangerous levels for consumers, the European Medicines Agency established the maximum residue limits for Ivermectin in the European Union, with values of 100 μgkg^{-1} in fat and liver and 30 μgkg^{-1} in kidney for all mammalian food-producing species. In India, the Food Safety Standards Authority of India (FSSAI) prescribes the residual limits of IVM in tissues, and maximum residue limits are available only for cattle, pigs, and

sheep, as per food safety and standards (contaminants, toxins, and residues) regulations, 2011.

Conclusion

The aquaculture sector is facing economic losses due to diseases caused by crustacean parasites. These parasites have developed resistance to commonly used anti-parasitic drugs, resulting in increasing economic losses. Ivermectin (IVM) is effective at low doses, has a broad range of activity against parasites, and provides long-term protection against infestation. At present very limited data is available on the pharmacokinetics, tissue level distribution, withdrawal period, toxicity of drug in fish and other environmental organisms. Further studies are required to establish the application of IVM in aquaculture at safe levels. According to the 2011 Food Safety and Standards Regulations (FSSR), there is no maximum residue limit (MRL) established for ivermectin (IVM) in fish muscle. Additionally, there is a lack of information regarding the recommended therapeutic dosage for treating parasitic infestations in fish. Therefore, it is essential to standardize the MRLs and therapeutic dosages of IVM to facilitate its future use in the aquaculture industry for controlling parasitic infections. This will help increase production and reduce economic losses associated with diseases caused by crustacean parasites.

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