African Journal of Food Science Vol. 4(9), pp. 578 - 584, September 2010
Available online http://www.academicjournals.org/ajfs
ISSN 1996-0794 ©2010 Academic Journals

Full Length Research Paper

Tissue distribution and elimination of erythromycin in giant freshwater prawn (*Macrobrachium rosenbergii*) depletion

N. P. Minh[1]*, T. B. Lam[1], N. T. Giao[2] and N. C. Quan[2]

[1]Department of Food Technology, Ho Chi Minh City University of Technology, 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City, Vietnam.
[2]VietNam - Russia Tropical Center, South Branch, 3 3/2 Streets, District 10, HCM City, Vietnam.

Accepted 10 August, 2010

Adult giant freshwater prawns (*Macrobrachium rosenbergii*) were fed with practical diets medicated with erythromycin (50 and 100 mg/ kg shrimp for 7 days), while erythromycin residues in their muscle were determined by LC-MS/MS method through TUV Rheinland Aimex Vietnam Co., Ltd. Interpolation of the study's data, following European Agency for the Evaluation of Medicinal Products Guidelines, gave a withdrawal time of 976°C-days (°C-day_water temperature-days) or 35 days. Our study provides preliminary data for a more prudent use of erythromycin in giant freshwater prawns, suggesting a possible withdrawal time after treatment. However, medication at a dose of 100 mg.kg^{-1} prawn body weight.day^{-1} for 7 days via feed, shows that erythromycin was derived slightly by erythromycin E (2.09 µg/kg) after 1 day of post-treatment. This biotransformation was kept on derivation of erythromycins (erythromycin E: 5.81 µg/kg, erythromycin F: 3.52 µg/kg) at a safe residue on day 23 of post-treatment.

Key words: Giant freshwater prawns, erythromycin, LC-MS/MS, withdrawal time, biotransformation.

INTRODUCTION

Giant freshwater prawn (*Macrobrachium rosenbergii*) has been considered one of the most important species of freshwater aquaculture in Vietnam, especially in the Mekong delta. Prawn culture in Vietnam comprised several models such as integrated and alternative culture with rice on rice paddy, semi-intensive culture in ponds and intensive culture in pen located along river/canal banks. Increasing demand of this species for domestic consumption and export markets has increased re-markably, scampi cultured systems with large scale, high stocking density and intensive feeding. Hence, disease is inevitable in these uncontrollable culture models. However, a variety of pathogens has been found in larval, juvenile and adult *M. rosenbergii*, including fouling proto-zoans such as *Epistylis, Zoothamnium* and *Vorticella* and pathogenic bacteria such as *Vibrio, Aeromonas, Pseudomonas, Edwardsiella*, etc. Bacterial necrosis is a common disease observed in adult prawns and has been termed variously as 'black spot', 'brown spot', 'shell disease' or chitinolytic bacterial disease. It is caused by the invasion of chitinolytic bacteria, which break down the chitin of the exoskeleton. Dat (2002) reported that *Aeromonas hydrophila, Aeromonas caviea, A. sorbia* and *Aeromonas sp.* are bacterial flora that isolated from necrosis prawns; meanwhile, *Edwardsiella tarda* and *Pseudomonas fluorescens* are two other species that isolated from adult prawns (Be, 2002). Moreover, *Aeromonas sp., P. fluorescens* and *Edwardsiella tarda* were bacteria flora that isolated from adult prawns (Tran et al., 2002). Gram-positive, ovoid and plococoid bacterium, tentatively identified as *Enterococcus*-like, was isolated from diseased *M. rosenbergiiin* Taiwanese aquaculture ponds. The diseased prawns displayed poor growth, anorexia, inacti-vity, opaque and whitish musculature and mortality. In histological preparations, melanized hemocytic granulomas were seen in the connective tissue around hemal sinuses together with hemocytic aggregation in necrotic musculature (Winton

*Corresponding author. E-mail: stapimex@gmail.com.

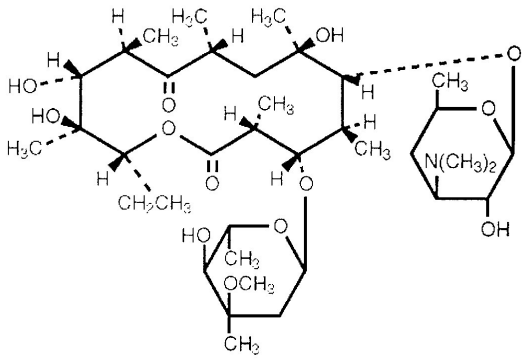

Figure 1. Chemical structure of erythromycin A.

and Jiam-Chu, 1998). Major groups of bacteria comprising the flora of *M. rosenbergii* were *Aeromonadaceae, Enterobacteriaceae* and *Pseudomonas*, while farmed *M. rosenbergii* carried pathogenic bacteria such as *Enterococcus* spp., *S. aureus, Aeromonas hydrophila, A. veronii* biovar *sobria* and *Clostridium perfringens* (Lalitha and Surendran, 2006). Shih-Chu et al. (2001) proved that *Lactococcus garvieae* infection in the giant freshwater prawn *M. rosenbergii* with opaque and whitish muscles were approximately 2 months old with total lengths from 5 to 6 cm. Histopathologically, they showed marked edema and necrotic lesions with inflammation in the muscles and hepatopancreas.

Be (2002) have documented that *Pseudomonas fluorescens* isolated from adult prawn was sensitive to erythromycin, whereas Dat (2002) conducted another study on the sensitive characteristic of isolated bacteria from necrosis prawns. The result showed that erythromycin was sensitive to four isolated species: *Aeromonas hydrophila, A. caviea, A. sorbia* and *Aeromonas sp.* However, Tran et al. (2002) showed that erythromycin could inhibit the growth of isolated bacteria such as *Aeromonas sp., A. hydrophila, A. sorbia* and *A. caviea.* Erythromycins are broad spectrum antibiotics that exhibit high activity against nearly all grampositive and gram-negative bacteria. Erythromycin A consists of a polyhydroxylactone and two sugars (Figure 1). Generally, erythromycin is the antibiotic of choice against *Aeromonas hydrophila, A. caviea, A. sorbia*, *Aeromonas sp.* and *Pseudomonas fluorescens.*

According to the regulation of Codex, WHO/FAO, EU, US, Canada, Australia, etc., erythromycin (Figure 2) residue in Seafood muscle must be lower than 30 ppb (Table 1). Vietnam Ministry of Agriculture and Rural development regulated erythromycin to a limited antibiotic with a maximum residual limit of 200 ppb. Farmers can use this substance in their farming, providing that they obey the withdrawal time. However, there are limited studies being published relating to accumulation and clearance of erythromycin in adult giant freshwater prawn.

The aim of the present study is to follow the uptake, depletion time and derivative metabolism of erythromycin in giant freshwater prawn muscle (*M. rosenbergii*) after oral administration of the drug given by the medicated feed. Moreover, a tentative withdrawal time was interpolated.

MATERIALS AND METHODS

Chemicals

Erythromycin base in white powder and purity (96.5%) was purchased from DHG Pharma (Can Tho, Vietnam).

Animals and diet

A total of 750 adult giant freshwater prawns (*M. rosenbergii*), with an average weight of 40 ± 2 g were used for the investigation. They were reared in farms (An Phu hamlet, Ke Sach district, Soc Trang province, Vietnam) at pH 7 - 8.5, an O_2 concentration of 5 - 6 mg liter^{-1} and an average temperature of 28 ± 0.5°C, without having toxic gases such as H_2S, NH_3, NO_2, CH_4, etc.

These 750 adult giant freshwater prawns were separated into two groups: Group A (375 prawns) and Group B (375 prawns) and two different diets were prepared for the experimental trial. Group A was treated with 50 mg kg^{-1} prawn body weight day^{-1} for 7 days through medicated feed (water temperature, 28°C), while Group B was treated with 100 mg kg^{-1} prawn body weight day^{-1} for 7 days through medicated feed (water temperature, 28°C).

Two groups of medicated feed were conditioned by weighing and mixing the feed with erythromycin base at appropriate dosages and a combination of drug and feed was adhesively guaranteed by a coating agent (squid liver oil).

The main parameter of this research was to investigate and demonstrate the difference of the egress pharmacokinetic of erythromycin A residue in prawn tissue between the low dosage and the high one. In the untreated control group, we could not see the distribution as well as clearance of drug, so there was no need of having an untreated control group in this experiment.

Temperature monitoring

Freshwater prawns are poikilothermic and the optimum metabolic temperature range for them is between 26 and 32°C. Temperature could have a strong effect on their survival and enzymatic metabolism, including drug biotransformation, so influence of temperature fluctuation at sampling time was recorded and mentioned in the drug metabolism calculation.

Sample collection and preparation

Erythromycin base material had been estimated previously to screen and confirm whether other derivatives of erythromycin A, such as erythromycin B, C, D, E and F have been available or not.

In each time of muscle sampling, 75 prawns were randomly sampled at 1, 3, 6, 9 and 23 days after 7 days of the pharmacological treatment. Meanwhile, bio-transformation of erythromycin in prawn was monitored by screening and confirming derivative forms of erythromycin at the beginning and the end of sampling stage. Muscle samples in natural proportion were collected and placed in polyethylene bags, coded and transferred to the laboratory on dry ice and analyzed within 8 h.

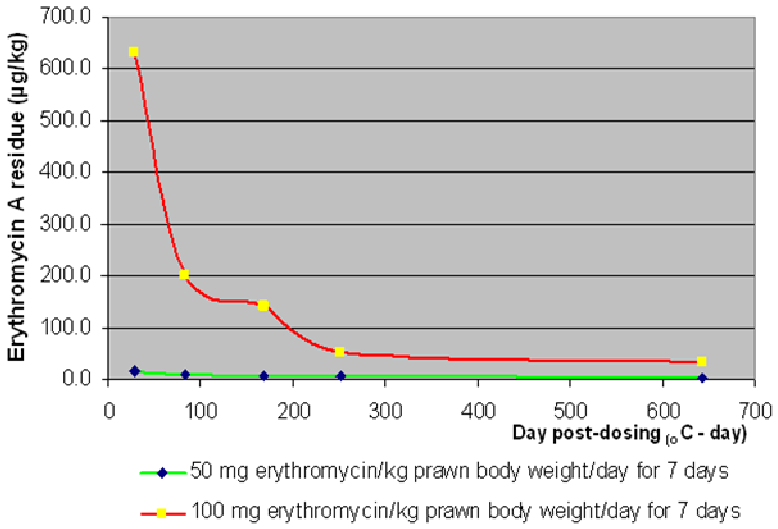

Figure 2. Erythromycin A depletion at different times in giant prawn muscles treated with 50 and 100 mg.kg^{-1} prawn body weight.day^{-1} for 7 days.

Table 1. MRL of erythromycin in foodstuffs regulated by importing markets.

Sample	MRL (ppb)					
	Codex (2008)	FAO/WHO (2006)	EU (2008)	US (2005)	Canada (2009)	Australia (2009)
Muscle	100	100	200	100	30	300
Liver	100	100	200	100	30	300
Kidney	100	100	200	100	30	300
Fat	100	100	200	100	30	300
Egg	50	50	150	25	30	300

MRL: Maximum residue limit.

Analytical procedures

The methodology used for the determination of erythromycin A, as well as derivatives of erythromycin in erythromycin base material, in prawn muscle was based on LC-MS/MS via TUV Rheinland Aimex Vietnam Co., Ltd. certified by DIN EN ISO/IEC 17025:2005 from DGA (German accreditation). Methanol as the extraction solvent, a temperature of 80°C, a pressure of 1500 psi, an extraction time of 15 min, 2 cycles, a flush volume of 150% and a purge time of 300 s were used in the analysis.

RESULTS

Tissue depletion

In order to consider the influence of water temperature on prawn metabolism and consequently, on the drug pharmacokinetics, the time parameter was also expressed as °C-day. Degree-days were calculated by multiplying the mean daily water temperature by the total number of days at which the temperature was measured to that point.

Results of erythromycin A depletion at different times in prawn muscle samples treated with 50 and 100 mg kg^{-1} prawn body weight day^{-1} for 7 days were shown in Tables 2 and 3, respectively.

Figure 3 showed some of the data reported in Table 3 that are displayed on a semilogarithmic graph, where a single animal data were plotted. On the y axis, erythromycin concentration (µg/kg) was plotted, while on the x axis, time post treatment (degree-days) was shown. The MRL value for erythromycin was set at 30 µg.kg^{-1}, as reported by CFIA (Canadian Food Inspection Agency) on 17/11/2009. The regression line and the upper one-sided tolerance limit (95%) regression line with a confidence of 95% were also traced. This graph had been obtained using the statistical program recommended by the European Agency for the Evaluation of Medicinal Products (EMEA) and was downloadable from the same EMEA web site. Using this statistical method, a withdrawal time of 976 °C-days was interpolated for giant freshwater prawn treatment, with 7 days of 100 mg.kg^{-1} prawn body weight.day^{-1} erythromycin.

Table 2. Erythromycin A depletion at different times in giant prawn muscles treated with 50 mg kg^{-1} prawn body weight day^{-1} for 7 days.

Time		Erythromycin A residue in prawn muscle (µg/kg)[α]
Day	℃ – Day	
1	28	15.4 ± 3.3
3	84	10.6 ± 2.1
6	168	5.9 ± 3.1
9	252	5.5 ± 4.1
23	644	2.8 ± 0.8

α Values shown are concentration means ± standard deviations from 5 prawn samples.

Table 3. Erythromycin A depletion at different times in giant prawn muscles treated with 100 mg kg^{-1} prawn body weight day^{-1} for 7 days

Time		Erythromycin A residue in prawn muscle (µg/kg)[α]
Day	℃ - Day	
1	28	632.4 ± 74.1
3	84	199.0 ± 31.2
6	168	141.8 ± 3.1
9	252	54.2 ± 9.0
23	644	31.4 ± 7.5

α Values shown are concentration means ± standard deviations from 5 prawn samples.

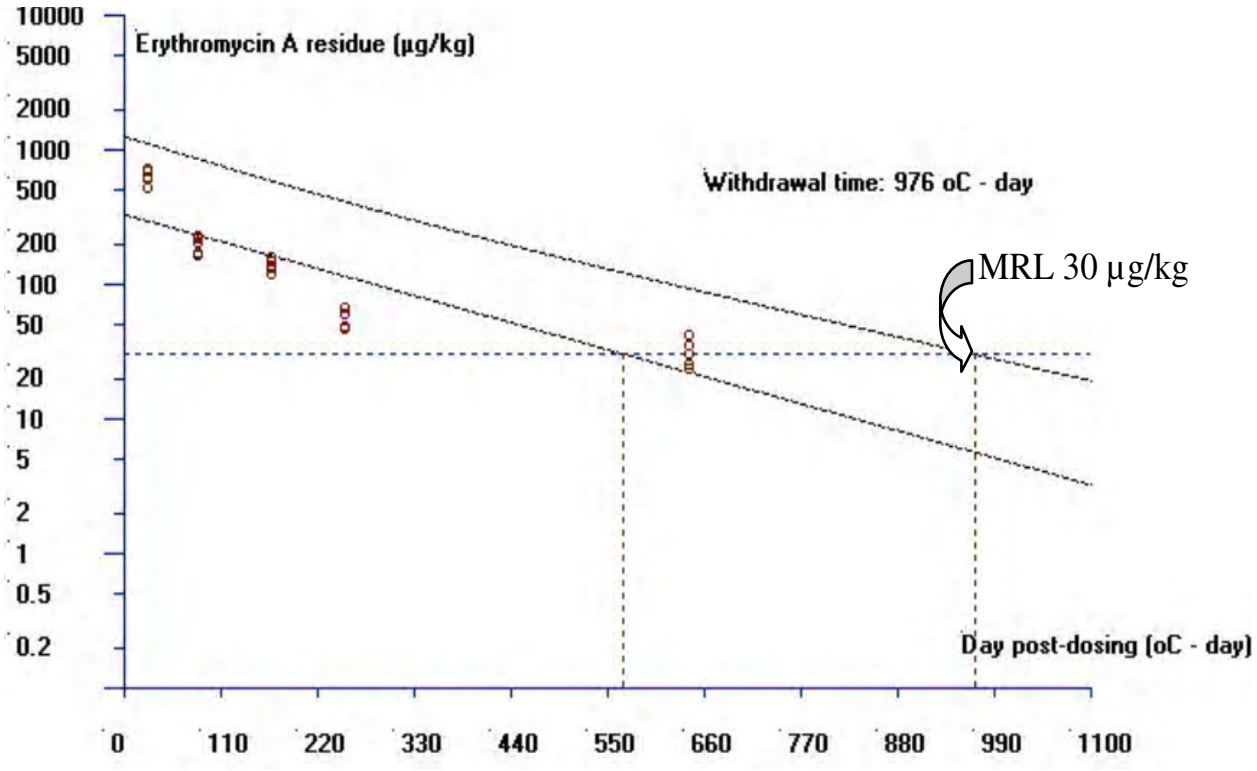

Figure 3. Linear regression line and upper one-sided tolerance limit (95%) linear regression line, with a confidence level of 95%, of erythromycin concentrations in muscle prawn treated with erythromycin for 7 days (100 mg kg^{-1} prawn body weight.day^{-1}) versus time. Degree-days are calculated by multiplying the mean daily water temperatures by the total number of days measured.

Table 4. Bio-tranformative forms of erythromycin at different times in giant prawn muscle samples treated with 50 mg.kg^{-1} prawn body weight.day^{-1} for 7 days.

Name of sample	Identification	Test parameter	MDL (µg/kg)	Result (µg/kg)
Erythromycin Base	EBS/0901-RC-002	Erythromycin B	1.0	N.D
		Erythromycin C	1.0	N.D
		Erythromycin D	1.0	N.D
		Erythromycin E	1.0	N.D
		Erythromycin F	1.0	5.00
Giant Prawn Muscle	GP - S1	Erythromycin B	10.0	N.D
		Erythromycin C	10.0	N.D
		Erythromycin D	10.0	N.D
		Erythromycin E	10.0	N.D
		Erythromycin F	10.0	N.D
Giant Prawn Muscle	GP - S5	Erythromycin B	10.0	N.D
		Erythromycin C	10.0	N.D
		Erythromycin D	10.0	N.D
		Erythromycin E	10.0	N.D
		Erythromycin F	10.0	N.D

* MDL: Method detection limit; **N.D: Not detected.

Table 5. Bio-tranformative forms of erythromycin at different times in giant prawn muscle samples treated with 100 mg.kg^{-1} prawn body weight.day^{-1} for 7 days.

Name of sample	Identification	Test parameter	MDL (µg/kg)	Result (µg/kg)
Erythromycin Base	EBS/0901-RC-002	Erythromycin B	1.0	N.D
		Erythromycin C	1.0	N.D
		Erythromycin D	1.0	N.D
		Erythromycin E	1.0	N.D
		Erythromycin F	1.0	5.00
Giant Prawn Muscle	GP - SC1	Erythromycin B	10.0	N.D
		Erythromycin C	10.0	N.D
		Erythromycin D	10.0	N.D
		Erythromycin E	10.0	2.09
		Erythromycin F	10.0	N.D
Giant Prawn Muscle	GP - SC5	Erythromycin B	10.0	N.D
		Erythromycin C	10.0	N.D
		Erythromycin D	10.0	N.D
		Erythromycin E	10.0	5.81
		Erythromycin F	10.0	3.52

* MDL: Method detection limit. ** N. D: Not detected.

Bio-transformation of parent drug

The bio-transformation of parent drug is explained in Tables 4 and 5.

DISCUSSION

Our research was designed in conditions that were quite close to actual aquaculture. Based on the results of our

Table 6. Formula of erythromycin A and related substances.

Erythromycin	Formula	Molecular mass	R_1	R_2	R_3	R_4	R_5
A	$C_{37}H_{57}NO_{13}$	734	OH	H	H	OCH_3	CH_3
B	$C_{37}H_{57}NO_{12}$	718	H	H	H	OCH_3	CH_3
C	$C_{38}H_{55}NO_{13}$	720	OH	H	H	OH	CH_3
D	$C_{36}H_{65}NO_{12}$	704	H	H	H	OH	CH_3
E	$C_{37}H_{67}NO_{13}$	748	OH	-O-		OCH_3	CH_3
F	$C_{37}H_{67}NO_{14}$	750	OH	OH	H	CH_3	CH_3

study, the mean concentration of erythromycin in group A was lower in comparison with that in group B. However, the eliminating slope of erythromycin residue in group B was faster than the one in group A.

Salmon *Oncorhynchus mykiss*, after its erythromycin administration at 100 mg.kg^{-1} trout body weight.day^{-1} for 21 days, through medicated feed (water temperature, 11.5°C), gave a withdrawal time of 255°C-days (Annarita et al., 2007), while the mechanism of Salmon *Oncorhynchus tshawytscha* retention and its depletion, through intraperitoneal injection (William, 2006), as well as orally administered erythromycin (Fairgrieve, 2005), was also investigated.

The digestive enzymes of tryptase, pepsin, cellulase, amylase and metabolic enzymes of alkaline phosphatase (AKP), acid phosphatase (ACP), superoxide dismutase (SOD) and glutathione-S-transferase (GST) were dominated in the hepatopancreas of *M. rosenbergii*, whereas only erythromycin F (5 µg/kg) was presented in erythromycin base. During medication at dose 100 mg.kg^{-1} prawn body weight.day^{-1} for 7 days via feed, erythromycin slightly changed to erythromycin E (2.09 µg/kg) after ceasing drug intake for one day. At day 23 of post-treatment, erythromycin E (5.81 µg/kg) and erythromycin F (3.52 µg/kg) were detected and fortunately, they were not significant to the study. Considering this, biotransformation would be able to keep erythromycin derivatives at a safe residue, if we tightly obey the recommendation of withdrawal time and drug dosage.

The aglycone part of all erythromycin molecules and the erythronolide is a 14-membered lactone ring, depending on the type of erythromycin this lactone ring is substituted via 4-position with a cladinose in the case of erythromycin A, B, E and F and with a mycarose in the case of erythromycin C and D (Table 6). However, all erythromycin molecules contain aminosugar d-desosamine, which is β-glycosidic linked to the 6 position of the lactone ring.

The minimum inhibited concentration of erythromycins A, B, C and D and some of their derivatives were determined against 21 gram-positive and 15 gram-negative microorganisms. Antibacterial activity was confined to gram-positive and very few gram-negative bacteria. Erythromycin B was somewhat less active than erythromycin A and erythromycin C and D showed about half that activity or even less. However, most other derivatives had negligible activity (Isaac, 1985).

There were lots of researches about toxicity, carcinogeneticity and genotoxiy of erythromycin A and erythromycin derivatives on mice, rat, dog and even on humans. However, researches about toxicity, carcinogeneticity and genotoxiy of erythromycin A and erythromycin derivatives in aquatic animals were scarcely investigated, especially on giant freshwater prawn. Biotransformative forms of erythromycin B, C, D, E and F were always in the mind of humans with the thought of whether they could be transformed from parental drugs during prawn aquaculture or not and whether or not they could create harmful risks to human health. So this research proved evidently that biotransformative forms of erythromycin obviously appeared through prawn enzymatic metabolism. This would set a basic foundation for upcoming researches about toxicity, carcinogeneticity and genotoxiy of erythromycin B, C, D, E and F.

Conclusion

Drug residue levels dropped quickly during the first 3 days after treatment termination and then, slowly and steadily until it gets to a residue level of < 100 µg/kg, which was considered a safe limit by FDA and the European Community requirements that was attained at day 9 of erythromycin withdrawal. Afterwards, a longer withdrawal period (35 days of post-treatment) was recommended to ensure complete drug depletion to safisfy the concern of CFIA.

However, the CFIA is opportune to underline the fact that, as a general policy, the use of antimicrobials which had an importance in human medicine, like erythromycin, should be limited to strictly necessary circumstances in veterinary medicine. The potential selection of erythromycin-resistant bacteria in aquaculture settings and the possible dissemination of such resistant clones and/or erythromycin resistance genes to humans might be hazardous for human health.

ACKNOWLEDGEMENTS

We deeply thank Dr. Nguyen Ba Hoai Anh from the

University of Science and Dr Nguyen Nhu Tri from Nong Lam University for their technical assistance.

REFERENCES

Ahmed H, Al-Harbi (2003). Bacterial flora of freshwater prawn, *Macrobrachium rosenbergii* (de Man), cultured in concrete tanks in Saudi Arabia. J. Appl. Aquat., 14:113 – 124.

Annarita E, Laura Fa, Dario L, Luigi M, Ettore C, Emilio G (2007). Orally administered erythromycin in rainbow trout (*Oncorhynchus mykiss*): residues in edible tissues and withdrawal time. Antimicrob. Agents Chemother., 51: 1043–1047.

Be LM (2002). Investigation on diseases of giant freshwater prawn (*Macrobrachium rosenbergii*) in ponds and rice-prawn farming systems in An Giang province. Msc. thesis (in Vietnameses).

Dat NT(2002). Investigation on parasite and bacteria diseases in giant freshwater prawns (*Macrobrachium rosenbergii*) cultured in pond and rice-prawn with low density. Msc. Thesis (in Vietnamese).

European Agency for the Evaluation of Medicinal Products. (2009). Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: marker residue depletion studies to establish product withdrawal periods. EMEA, London, United Kingdom. http://www.ema.europa.eu/pdfs/vet/vich/46319909en.pdf

Fairgrieve WT, Masada CL, McAuley WC, Peterson ME, Myers MS, Strom MS (2005). Accumulation and clearance of orally administered erythromycin and its derivative, azithromycin, in juvenile fall Chinook salmon *Oncorhynchus tshawytscha*. Dis. Aquat. Organ., 64: 99-106.

Isaac OK, Jos H, Eugene R, Hubert V, Ludo V, Maurice D, Claude PV, Pierre P, Georgette L (1985). Antibacterial activities of erythromycins A, B, C, and D and some of their derivatives. Antimicrob. Agents Chemother., 28: 630-633.

Lalitha KV, Surendran PK (2006). Microbiological quality of farmed tropical freshwater prawn *(Macrobrachium rosenbergii).* J. Aqua. Food Pro. Technol., 15: 71 – 82.Na Li, Yunlong Z, Jian Y (2008). Effects of water-borne copper on digestive and metabolic enzymes of the giant freshwater prawn *Macrobrachium rosenbergii.* Archives of Environ. Contam. Toxicol., 55: 86-93.

Shih-Chu C, Yu-De L, Li-Ling L, Pei-Chi W (2001). *Lactococcus garvieae* infection in the giant freshwater prawn *Macrobranchium rosenbergii* confirmed by polymerase chain reaction and 16S rDNA sequencing. Dis. Aquat. Organ., 45: 45-52.

Tran T, Tuyet H, Dang T, Hoang O, Nguyen TP (2002). Study on Diseases in giant freshwater prawns (*Macrobrachium rosenbergii*): A review.

William T, Cyndy L, Masada ME, Peterson W, Carlin M, Gail CM, Mark SS (2006). Concentrations of erythromycin and azithromycin in mature Chinook salmon *Oncorhynchus tshawytscha* after intraperitoneal injection, and in their progeny. Dis. Aquat. Organ., 68: 227 – 234.

Winton C, Jiam-Chu C (1998). Isolation and characterization of an *Enterococcus* like bacterium causing muscle necrosis and mortality in *Macrobrachium rosenbergii* in Taiwan. Diseases of Aquatic Organisms, 34: 93-101.