Egypt. J. Agric. Res., 78 (1) 2000.

EFFICACY AND RESIDUES OF OXOLINIC ACID IN *OREOCHROMIS NILOTICUS* FISH INFECTED WITH ENTERIC RED MOUYH DISEASE (ERM)

MONA M. HUSIEN AND A.A. YOUNIS

Animal Health Research Institute, Agricultural Research Centre, Dokki, Giza-Egypt.

(Manuscript received 6 January 1999)

Abstract

Efficacy and residues of oxolinic acid in tissues of experimentally inoculated *Oreochromis niloticus* fish with *Yersinia ruckeri* (*Y.ruckeri*) have been investigated. Results of the in-vitro stuy revealed that the used bacterial isolate was sensitive to oxolinic acid.

The in-vivo treatment test indicated a high effectiveness of oxolinic acid when given orally in the diet at a dose rate of 10 mg/kg body weight for ten successive days.

The haematological inestigations in experimentally infected fish proved a decrease in some blood parameters namely, RBCs, WBCs count, Hb content and P.C.V. volume pre-treatment with oxolinic acid, but they returned to their normal values post-treatment. The residual analysis indicated the presence of oxolinic acid residues in the muscles, kidney and liver of treated fish up to 15, 20 and 25 days post-treatment, resppectively.

INTRODUCTION

Enteric Red Mouth disease is an acute to chronic bacterial infection which affects mainly the salmonid fishes with severe economical loss (Davies 1990). Other fish species can be affected (Inglis *et al.*, 1993). *Y.ruckeri*, the etiological agent of ERM was isolated from *O.niloticus* fish in Egypt (Mona *et al.*, 1997). Morbidity of ERM may reach to 100% at any age of fish, while, the mortality is usually higher in younger fish (Post, 1997). Recently, the disease caused by *Y.ruckeri* has been referred to as yersinosis or yersinia septicemia as the classic red mouth is not necessary seen (Inglis *et al.*, 1993). Control of clinical cases of ERM may be medicated by antimicrobial compounds and chemotherapy. Sulphamerazine and/or oxytetracycline has been advocated (Rucker, 1966) but there is evidence that certain bacteria which are pathogenic for fish can acquire

plasmide mediated resistance to the type of drug (Aoki *et al.*, 1977). Recent work has highlighted the value of oxolinic acid for the control of yersinosis (Rodgers and Austin, 1982). Oxolinic acid showed high efficacy against many Gram-ve bacterial fish pathogens and it is well absorbed orally (Alderman, 1988).

The present study was planned to follow up the efficacy and tissue residues of oxolinic acid in *O.niloticus* fish experimentally infected with *Y.ruckeri*, also, to measure the changes in some blood parameter of infected *O.niloticus* before and after treatment with oxolinic acid.

MATERIALS AND METHODS

1. Fish

A total of 120 apparently healthy alive *O.niloticus* fish with an average body weight of 80±10g were collected from Nawa farm at Kalubia Governorate to be used in experimental infection. Fish were kept in glass aquaria (60x80x100 cm) supplied with aerated chlorine-free tap water at 25±1°C (Inness, 1996). Fish were fed commercial ration twice a day to satiation and left 2 weeks for acclimatization. Thirty fish from each were used for determination of LD50, while, the remained 90 fish were used in experimental infection.

2. Bacterial isolate

1. In this study, a complete identified locally isolated *Y. ruckeri* (Mona *et al*, 1997) was used. It was propagated on trypticase soy broth to be used in the experimental inoculation of fish and on trypticase soy agar (oxoid) to be used in the vitro sensitivity testing.

2. *Bacillus subtilis*

This was used as standard organism (obtained from Animal Health Research Institute) for detection of oxolinic acid residues in fish tissue.

3. Drug

1. Oxolinic acid : (Urotrate), Obtained as tablets containing 750 mg oxolinic acid Park-Davis, France.

2. Tricaine methane sulphate: (Ms-222) Sandoz, was obtained as powder and was ued as fish anaethetic at a dose of 50 mg/L (Stoskopf, 1993).

4. The in-vitro inhibitory activity of oxolinic acid

According to Austin, *et al.* (1981), the isolate of *Y.ruckeri* used was incubated for 7 days at 22°C on tryptone soy agar (oxoid) supplemented with 0.5, 1.0, 1.5, 10,50 and 100 mg/L of oxolinic acid for determination the in vitro inhibitory activity of oxolonic acid.

5. Preparation of medicated feed

a. Oxolinic acid was used at a rate of 10 mg/Kg body weight for treatment of *O.niloticus* experimentally infected with *Y.ruckeri* (Rodgers, *et al.*, 1983).

b. The pellets used as fish feed were ground and thoroughly mixed with determined dose of oxolinic acid, then, wetted with water and remanufactured into suitable size pellets using pellets using pellet machine. (El-Banna, 1991).

c. Fish were fed a ration of 3% of their body weight (Meske, 1985) twice daily.

6. Detection of oxolinic acid residues

Tissue samples were assayed by the microbiological method for estimating oxolinic acid qualitatively (Levetzew, 1971.). Piceces of liver, muscle and kidney of both treated and control fish were placed over the surface of nutrient agar media inoculated with *Bacillus subtilis* spore as standard organism at antibiotic medium No. 1 (El Nasr Pharmaceutical Chemical Co.). The inhibition zone around the tissue samples was measured; 2mm or more was considered positive result indicating the presence of antibiotic residue, 1mm or less was considered negative result indicating the absence of atibiotic residue (Arret *et al.* 1971).

7. Determination of LD_{50} of *Y.ruckeri* isolate

This was applied according to Reed and Menuch (1938). Thirty *O.niloticus* fish, were divided into 6 groups (5 fish/group). Fish in each group were injected I/M by 0.1 ml of bacterial dilution, 10^1, 10^2, ..., 10^6, of which the bacterial cell count of original sample was $2.5x10^8$ cell/ml.

8. Experimental design

In this study, a total of 90 apparently healthy normal *O.niloticus* with an average body weight (80±10 g) were divided into 3 groups of 30 fish each. All fish were anaesthetized and fish in group 1 and 2 were injected sub/cut with 0.5 ml of 24 h old broth culture containing $2x10^2$ cell/ml of *Y.ruckeri*, while, each fish in

group 3 was injected sub/cut with 0.5 ml/fish of sterile saline and kept as control. Fish were observed for the development of clinical sings indicating *Y.ruckeri* infection. After the appearance of clinical signs in experimentally infected group, (72 h post-infection), ten blood samples were randomly collected from each group for haematological examination according to Lucky (1977). After that, the fish in the fist group were fed on medicated diet (10 mg of oxolinic acid/Kg body weight) for 10 days according to Collins *et al.* (1996), while, fish in the other groups were fed on medicated free diet, 72 h after the end of treatment. Other 10 blood samples were randomly collected from the first group. Blood samples were used for estimating total erythrocytic counts, packed cell volume (Haematocrite) haemoglobin percentage and total leucocytic counts according to the method desribed by Lucky (1977).

For studying the residues of oxolinic acid, 3 samples were taken from muscles, kidney and liver from fish in group 1 after killing at the rate of 5 days intervals through 30 successive days. A positive result was indicated by the complete inhibition of growth of the tested organism in anular zone not less than 2 mm width (Levetzew, 1971 and Anon, 1980).

RESULTS AND DISCUSSION

The results of LD_{50} of *Y.ruckeri* isolate carried out according to Reed and Menuch (1938), indicated that LD_{50} value for *Y.ruckeri* isolate was $2.5x10^6$ cell/ml. Studying the in-vitro inhibtory activity of oxolinic acid revealed that the *Yersinia ruckeri* isolate was sensitive to 5 mg/l. These findings concide with Austin, *et al.* (1981) and Collins, *et al.* (1996).

Efficacy of oxolinic acid

The most pronounced clinical signs of the experimental *Y.ruckeri* infection on *O.niloticus* fish started through the first 48 h post-infection. Infected fish showed sluggishness and darkness in colour (Fig. 1), petechial haemorrhage or haemorrhagic body surface, tail and fins be eroded and erythema in the mouth region. The moribund fish percentage was 60% in the first two groups (infected groups), while, mortality rate was low (10%) (Table 1). In case of group 1 (treated group), clinical signs indicating *Y.ruckeri* affection started to relieve within 96 h post-fed on medicated diet with oxolinic acid. Fish returned to their normal activity, signs of septiceamia declined, the mortality rate was stopped. During the reisolation, *Y.ruckeri* disappeared from internal organs of treated fish, while, it was isolated from internal organs from non-treated fish (group 2), in which the lesions progressed to haemorrhagic patches allover the body, particularly around the base

of the fins and in the head regions (Fig. 2). In some fish, the anus was protruded and oedematous with redness in the perianal tissue. At the end of experiment, the morbidity rate reached to 90%, while, mortality reached 40%. These findings concide with those of Wobsere (1973). Results of in-vitro study confirmed the previously mentioned results of the effect of oxolinic acid on infected fish with *Y.ruckeri*.

Haematological findings

It appears from Table 2 that, the experimental infection of *O.niloticus* with *Y.ruckeri* resulted in significant decrease in red blood cell counts, haematocrite values, haemoglobin content and total leucocytic count. These findings were also in agreement with those reported by Quentel and Aldrin (1986) and Lehmann *et al.*, (1987). Miller, (1983) suggested that the endotoxin of *Y.ruckeri* could affect blood coagulation, ultimately producing thromobsis in the capillaries and haemorrhages. However, all examined parameters approached to their normal limits after the course of medicated feed, but they still showed non-significant decrease than their normal limits.

Oxolinic acid residues

The residues of oxolinic acid in experimentally infected fish group fed on the medicated ration were determined qualitatively as shown in Table 3. It is clear that, oxolinic acid residues were detected in muscles up to 15 days,in kidney up to 20 days and finally up to 25 days in liver post-treatment. The presence of drug residues in kidney and liver more than muscles may be attributed to the fact that, kidney is the last gate allowing passage of drug before excretion, and liver plays the major role for detoxication and storage of the drug.

It could be concluded that, the use of oxolinic acid in ration can overcome the carrier stae of *Y.ruckeri* which serves as an important souce of infection under stress condition. We can also conclude that, fish treated with the drug should not be used by the consumer before 25 days following treatment to avoid resistance.

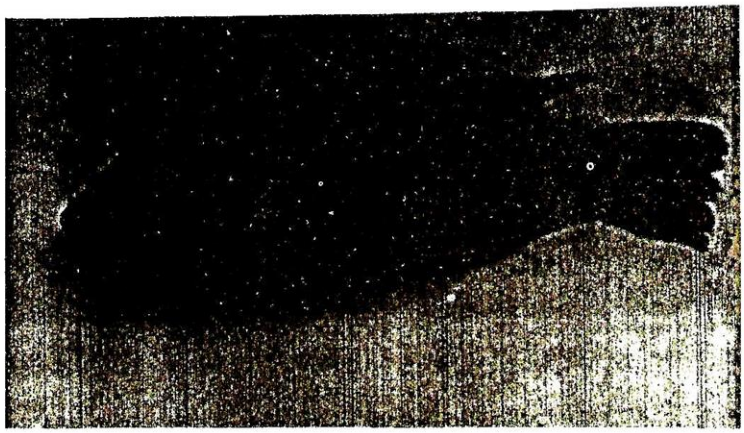

Fig.1. *Orechromis niloticus* experimentally infected with *Y. ruckeri* 96 h. post-ifection showing darkening in colour

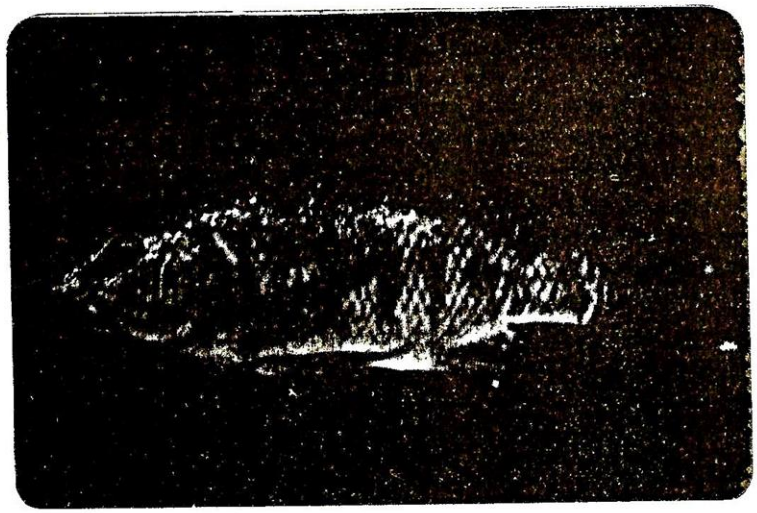

Fig.2. *Orechromis niloticus* experimental y infected with *Y. ruckeri* 5 days post-infection showing redness around mouth, eyes and perianal tissue

Table 1. Effect of oxolinic acid on the morbidity and the mortality rate of *O. niloticus* sub/cut injected with 0.5 mml of 2x 10^6cell/ml *Y.ruckeeri*

Fish groups	Type and duration of treatment	Morbidity percentage		Mortality percentage	
		before treatment	after treatment	before treatment	after treatment
1 (treated)	fed on diet contain 10 mg oxolinic acid / body weight for 10 days	60	10	10	10
2 (non treated)	fed on diet free from oxolinic acid	60	90	10	40
3 (control)	fed on diet free from oxolinic acid	-	-	-	-

Table.2. Haemtological picuture of *O. niloticus* fish infected with *Y. ruckeri,* before and after medication with oxolinic acid.

Groups	Haematological parameters			
	RBCs10^6/mm^3	Hb gm/dl	PCV%	WBCs 10^3/mm^3
infected fish before treatment	1.1 ± 0.20*	6.6 ± 0.42*	23.8 ± 0.86*	4.2 ± 0.16*
infected fish 10 day after medicated ration	1.84 ± 0.62	7.0 ± 0.52	24.4 ± 1.2	4.9 ± 0.38
Control	1.9 ±.0.22	7.9 ± 0.22	26.4 ± 0.95	5.2 ± 0.32

Significant at $P<0.05$

Table 3.Oxolinic acid residueds in *O. niloticuss* fish.

Groups	treated sample	Days post treatment					
		5	10	15	20	25	30
Oxolinic acid treated group	Muscle	3/3	2/3	1/3	0/3	0/3	0/3
	Kidney	3/3	2/3	1/3	1/3	0/3	0/3
	Liver	3/3	3/3	2/3	1/3	1/3	0/3
Control	Muscle	0/3	0/3	0/3	0/3	0/3	0/3
	Kidney	0/3	0/3	0/3	0/3	0/3	0/3
	Liver	0/3	0/3	0/3	0/3	0/3	0/3

The results are expressed as the number of samples with positive inhibition in relation to the number examined

REFERENCES

1.Adrman, D.J 1988. Fisheris chemtherapy: a review in Muir JF Roberst RJ:Recent advances in aquaculture, vol. 3 London. Croom Helm., pp.1-16.

2.Anon, W. 1980. A standardized method for the detection of residues of ntibacterial substances in fresh meat. Fleisswirtsh, 60:672-673.

3.Aoki, T., T. Arai and S. Egusa. 1977. Detection of R plasmids in naturally occuring fish-pathogenic bacteria, Edwadsiadsiella tarda. Microbiologia et Immunologia (Scandinavia), 21:77-85.

4. Arret, B., D.P. Johnson and A. Kirshbioem. 1971. Outline of details for microbiological assay of antibiotics. Pharmacological Sci.,60 (11) : 1689-1694.

5. Austin, B., D.A. Morgan D.J. 1981. Oxolinic acid for control of enteric red mouth disese. Aquaculture, 26 (1) 277.

6. Cipriano, R. C. W.B., S.W. Pyle and R Hornoi .1986. An epizootic in Chinook salmon caused by *Y.ruckeri.* T. Wild Dis., 22:488- 492.

7. Collins, R. O., G. Foster and H. M. Ross. 1996. Isolation of *Y.ruckeri* from on otter and salmonid fish from adjacent fresh water catchments. Vet. Rec., 139-169.

8. Davies, R.L. 1990. O.serotyping of *Y.ruckeri* with special emphasis on European isolates. Bet. Microbiol., 22:200-307.

9. El-Banna, R. A. 1991. Studies on some nutrients requirement for Tilapia and its effect on performance. Thesis, Ph. D., Fac Vet. Med. Cairo, University.

10. Inglis, V., R.J. Roberts and Bramage N.R. 1993. Bacterial Diseases of fish. Blakwell, scientific publications, London, Edinburgh Basten.

11. Innes, W.T. 1966. Exotic aquarium fishes. 19 th Ed. P.M. 12,24 (29). 530.

12. Lehmann, J., F.J. Sturenberg and D. Mock .1987. The changes in haemorgram of rainbow trout (Salmo gairdneri, Richardson) to an artificial and natural challenge with Y. ruckeri. J. Applied Iclhyology, (3): 147-183.

13. Levetzew, R. 1971. Studies of inhibitory substanes within the farmwork of bacteriological inspection of meat. Bundes uindheitsblatt, (14): 211-213.

14. Lucky, S. 1977. Methods for diagnosis of fish Glem L. Hoffman (Editor) Fish and wild life service, New York, USA.

15. Meskę, C. 1985. Fish Aquaclture Technology and Experiments Edited by Frederic vogl. Pergamon press.

16. Miller, T. 1983. Blood coagulation in E. R. M. infected trout: Role of bacterial endotoxin. Proceeding 8 th Ann. FHSIAFS Workshop, Kearneysville, Wv, pp. 48.

17. Mona, H.H., R.H. Nahla and E.M. Riad. 1997 Studies on enteric red mouth disease (ERM) among fresh water fish. Vet. Med. J., Giza, 45,(4):549-559.

18. Quentel, C. and J.F. Aldrin. 1986. Blood changes in characterized rainbow trout (Slmo gairdneri) in traperitoneally inoculated with *Y.ruckeri.* Aquaculture, 53:169-185.

19. Post, G. 1987. Textbook of fish Health. T.F. H. puplications, Inc. for Revised and expanded Edition

20. Reed, L.J. and H. 1938. A simple method of estimating fifty percent end point. Amer. J. Hyg., 27 (3): 493-497.

21. Rodgers, C.J. and B. Austin. 1983. Oxolinic acid for control of enteric red mouth disese in rainbow trout. Vet. Rec, 112: 83.

22. Rucker, R. 1966. Red mouthdiseas of rainbow trout (Salmo gairodneri). Bull. office Int. Epizoo., 65:825-830.

23.Stoskpf, M.K. 1993. Fish medicine.W. B. Saunderss Comparny, Harcout, Braze Jovanvoich, Inc. London.

24. Wobser, G. 1973. An outbreak of red mouth disease in rainbow trout (Salmo gairfneri) in Sasktchewan. J. fish. Res. Broad of Canada, 30:571-5705.

كفاءة ومتبقيات حامض الأوكسولنيج فى علاج أسماك البلطى المصابة تجريبيا بميكروب اليرسينيا روكرى

منى مصطفى حسين و عباس يونس

معهد بحوث صحة الحيوان، مركز البحوث الزراعية -وزارة الزراعة الدقى - جيزة - مصر.

تم دراسة تأثير وبقايا حامض الأوكسولينيج فى سمك البلطى المصاب بميكروب اليروسينيا روكرى. وأفادت الدراسة المعملية أن ميكروب اليروسينيا روكرى حساس لحامض الأوكسولينيج . وعند علاج السمك به باعطائه عن طريق الفم اختفت الأعراض المرضية فى الأسماك المصابه. وبقياس عدد كرات الدم الحمراء والبيضاء ونسبة الهيموجلوبين ونسبة الترسيب الدموى في الأسماك المصابة نقصت عن معدلها الطبيعى أثناء الأصابة بالعدوى التجريبية وعادت الى طبيعتها بعد استخدام العلاج. وأيضا أثبتت الدراسة أن متبقيات الحامض قد أفرزت من اعضاء الأسماك المعالجة بعد ١٥ ، ٢٠، ٣٠، يوما من العضلات، الكلية والكبد على الترتيب.