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COMPARISON OF EFFICACY OF FLORFENICOL IN REDUCING MORTALITY DUE TO *Yersinia ruckeri* INFECTION IN RAINBOW TROUT *(Oncorhynchus mykiss)* FRY

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Abstract

During the spring of 2006 unusuall mortality outbreaks among rainbow trout fry (7 – 10 cm in length) occurred in three separate ponds from a farm in a region of Tonekabon city in Iran, resulting in mortality of 36.50% (range 29.79 to 49.73). Enteric redmouth disease (ERM), caused by *Yersinia ruckeri* was diagnosed in rainbow trout fry. This disease is characterized by the presence of hemorrhages around the mouth and anus, at the base of the fins and on the surface of internal organs as confirmed by bacteriology. The aim of this study was to compare the efficacy of florfenicol (FF) in reducing mortality of rainbow trout fry with ERM disease. Efficacy was assessed by total mortality at the end of the treatment period and three days after withdrawal medication in which losses ceace. For this purpose, three ponds of the affected farm with a total of 5,328 rainbow trout fry were utilized. 1,752 fry in treatment group that received FF (10 mg/kg/day) medicated feed for 10 consecutive days and 1,859 in the positive control group that received oxytetracycline (OTC, 50 mg/kg/day) medicated feed for 10 consecutive days, and 1,717 in the negative or unmedicated feed group that no received any drugs. Results of trials indicated that a florfenicol medicated diet given to rainbow trout fry for 10 consecutive days resulted in effective control of ERM. There were significant differences between FF and OTC groups when compared with unmedicated group in reducing mortalities at the end of the treatment period ($p<0.001$ and $p<0.01$; respectively); however, there was no significant difference between the FF and the OTC groups at this time ($p>0.05$). The FF and the OTC groups were significantly more effective than unmedicated group in reducing mortality of the rainbow trout fry with ERM disease. The majority of deaths due to ERM disease occurred during the first day of period experiments. The FF reduced mortality to a lower level when compared to positive and negative control groups at the end of third days after withdrawal medication ($p<0.01$ and $p<0.001$; respectively). Overall specific mortality was 0.11% for FF group, 0.69% for OTC group and 1.51% for unmedicated group in third days after withdrawal medication.

Keywords: Florfenicol, Oxytetracycline, *Yersinia ruckeri* and Enteric redmouth disease (EMR).

1. Introduction

This Enteric redmouth disease (ERM) or Yersiniosis, caused by *Yersinia ruckeri*, is a systemic bacterial infection of salmonids and other fish species (Dear, 1988; Willumsen, 1989; Hietala *et al*., 1995). The disease has now been reported in most countries where trout are cultured (Roberts, 1983; Rigos and Stevenson, 2001). This pathogen has been reported in Iran (Jeremy and Teresa, 2002). EMR is characteristically a disease of rising and falling water temperatures. The highest risk of disease occurs between 8°C and 16°C. At the higher temperatures mortality

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can rise quickly. It is especially severe among intensively cultured rainbow trout (*Oncorhyncus mykiss*) reared in water more than 13°C (Dulin et al. 1976); although mortality can occur at temperatures as low as 4°C. The incubation period of infection is 5 to 10 days (Busch, and Lingg, 1975; Roberts, 1983). Fish most at risk are those subject to stress factors arising from poor management or environmental changes such as elevated temperature or poor water quality (Rodgers, 1991). This disease is now endemic in all trout producing countries where it can cause severe economic losses (Rucker, 1966; Ewing *et al*., 1978) and also, it is sometimes a problem in salmon aquaculture (Fuhrmann *et al*., 1983).

EMR disease can affected all age classes of fish but is most acute in small fish up to fingerling size. Several workers reported that fish size affects the susceptibility of fish (Rucker, 1966; Dulin *et al*., 1976), while the disease is seldom a problem in rainbow trout less than 7.5cm in length. However, size alone may not be the determining factor. It was reported that the number of fish affected is small in Atlantic salmon smolt usually 3-6 weeks after their introduction to sea water (0.1-0.75% per week) (Jeremy and Teresa, 2002). In the present study, the size of rainbow trout fry was 7-10 cm in length.

Yersinia ruckeri is a motile, Gram-negative, rod-shaped, fermentative, peritrichously flagellated bacterium (Ewing *et al*., 1978; Fuhrmann *et al*., 1983; Petrie *et al*., 1996). This pathogen is readily transmitted from fish to fish by contact and through the water (Rucker, 1966). Where ERM has become established, it usually causes sustained low-level losses. Occasionally, severe epizootics occur with mortalities exceeding 50% if corrective measures are not taken (Ross *et al*., 1966; Bullock *et al*., 1976; Altinok *et al*., 2001). Surviving fish frequently become asymptomatic carriers which can spread the disease if these fish are transferred to new locales (Busch and Lingg, 1975; Hunter *et al*., 1980). Enteric redmouth disease can be successfully controlled by a combination of environmental improvement and antibactetial drugs (Rucker, 1966; McDaniel, 1971).

Florfenicol (FF) is a synthetic, broad spectrum antibiotic active against many Gram-negative and Gram-positive bacteria isolated from animals. FF is both bacteriostatic and bactericidal antibiotic. Its activity is due to the inhibition of protein synthesis and results from the binding to the 50s of bacterial ribosomal subunit in such away as to prevent ongoing translation of mRNA into protein (Cannon *et al*., 1990). The absorption, distribution, metabolism and excretion of florfenicol have been studied in rainbow trout and Atlantic salmon in freshwater and seawater (Martinsen *et al*., 1993; Horsberg *et al*., 1994; Horsberg *et al*., 1996; Pinault *et al*., 1997). Conclusion from these studies was consistent for two species of fish in that florfenicol was well absorbed, and excreted in bile, feces and urine. The FF is highly palatable, well tolerated by fish (Inglis *et al*., 1991).

2. Materials and Methods

This study carried out in three 2500 liter separate round concrete ponds (2 meter in diameter, 1.5 meter in height) (Fig.1), at an average temperature of 15 -16ºC. Water flow in each pond was set at 12 l/min. During the investigation period, the pH and dissolved oxygen of the water in three ponds were uniform (pH 7.2-7.5 and dissolved oxygen 7-7.5 mg/l). The fish fry were fed eight times daily with a commercial trout feed (Behparvar Company). A total of 5,328 rainbow trout fry, weighing 5 - 6 gram and 7 – 10 cm in the total length were utilized in three separate ponds, with 1,752 fish in treatment group that received florfenicol medicated feed and 1,859 in the positive control group where they were medicated with oxytetracycline, and 1,717 in the negative or unmedicated feed group.

The Flora-Behrood (FF 10%), oral solution 200 ml, (Behrood Pharmaceutical Company) and oxytiline (OTC 20%), water soluble powder (Damloran Pharmaceutical Company) were purchased from local veterinary pharmacy. Each

ml of flora-Behrood was contained 100 mg florfenicol. The FF and OTC were used 1 ml (100 mg)/10 kg/b.w., and 50 mg/kg/b.w., respectively. Each of the drugs were added to 2 ml of liquid sunflower oil and 2 ml of distilled water; and after shaking, they were mixed with feed before fish feeding. The negative control group did not receive any drug; but, the identical volume of liquid sunflower oil and distilled water were added in its feed. The losses were collected and numbered in every morning before use of the drugs. The drug therapy was started in third days of outbreaks disease after determination ERM disease. The drugs were administrated once daily (9:00 AM), for 10 consecutive days. Losses were calculated the 10 days of period treatment and the 3 days after the end of period treatment. Lethargic and live fry were necropsied and macroscopic and microscopic findings were determined.

2.1. Statistical analysis

The data expressed s a mean ± SEM were statistically analyzed by the analysis of variance (ANOVA) followed by the Kruskal-Wallis nonparametric test for between group differences and Dunn's correction of the significance level for multiple comparison. The level of significance adopted was $p<0.05$. The calculation was performed on a personal computer, using the Instat program.

3. Results

In April 2006, ERM disease was diagnosed in rainbow trout fry in a region of Tonekabon city in Iran. Sudden change in water quality in due to the neglect and releasing of remained hogwash of hatchery hall into the ponds was believed to have contributed to unfavorable water quality as a trigger for the incident. Fish were maintained in three 2500 liter round concrete ponds at an average temperature of 15-16ºC. Three ponds containing 5,328 rainbow trout fry were most severely affected by sudden deaths. Laboratory investigations of live fry from the affected ponds resulted in the isolation of *Yersinia ruckeri* from kidney tissue. Bacteria were subcultured on trypticase soy agar (TSA) to check purity, and then cultured in trypticase soy broth (TSB) for 24 hrs at 22°C. Cumulative losses were of 1,934 over a 13-day period from total stock in the three ponds on 5,328. The worst affected pond experienced losses of up to 184 fry a day.

Generally, the first signs of disease were seen as an increase in mortalities above the normal attrition rate (Fig.2). Cumulative losses were of 451 fry in the first day of treatment and reached of 44 fry at the end of tenth days of period treatment (Fig. 3). Behavior changes in affected fry were swimming at the water surface, moving sluggishly, darkening, and reluctant to eat. Subcutaneous hemorrhages were observed in and around the mouth, oral cavity (Fig.4), in around the anus, and at the base of the pectoral and pelvic fins. Gill filaments were hemorrhagic. Bilateral exophthalmos often with frank patches of hemorrhagic congestion occurred on the iris of the eye (Fig.5). The affected eyes were often ruptured. Affected fry were often blind with little avoidance reaction. The posterior intestinal tract was inflamed and filled with a thick, yellowish fluid. The victims often had enlarged, friable, and dark spleens, hemorrhagic specks on the pyloric cecae, liver and adipose tissue. The stomach and pyloric caeca contained clear mucus. In atypical infections that sometimes were occurred, no hemorrhages were not developed on the mouth and gill cover; fry merely became dark and swam near the surface.

In this study, drug therapy was started with FF and OTC after determination ERM disease. The majority of deaths due to ERM disease occurred during the first day of medication (8.46%). Cumulative losses decreased from the 2nd day of medication and reached 8.08%. This value was 0.82% at the end of period treatment and reached 0.09% at the end of the third days after withdrawal medication (Fig.6). Cumulative losses were 36.50% (29.79% for FF, 30.00% for OTC and 49.73% for unmedicated group) at the end of duration experiments. Overall mortality was 522 for FF group, 558 for OTC group, and 854 for unmedicated group in duration experiments (Fig.7). Overall specific mortality was 0.11% for

FF group, 0.69% for OTC group and 1.51% for unmedicated group at the end of the third days of withdrawal medication (Fig.8).

4. Discussion

Enteric redmouth disease can develop fast or slowly. The quick cases (acute phase) are indicated by high mortality rate without apparent external symptoms. The slow or gradual form of this disease is more common, with considerable or moderate mortality rate for a few weeks, when losses abate. Mortality due to ERM disease in rainbow trout usually takes place in 5 to 10 days depending on the size of the fish, stress factors arising from poor management or environmental changes such as elevated temperatures or poor water quality and the general health (11 - 13). However, if the population has had prior exposure to and latent infection with *Yersinia ruckeri*, a stressful environment can result in mortality within 3 to 5 days (Bush, 1982).

Stressful condition such as poor water quality will increase the level of susceptibility and mortality. In the present study, sudden change in water quality in due to the neglect and releasing of remained hogwash of hatchery into the ponds was believed to have contributed to unfavorable water quality as a trigger for the incident. Several antibacterials including sulfamerazin (Dulin *et al*., 1976), oxytetracycline, erythromycin, quinolones (Ceschia *et al*., 1987), and the potentiated sulfonamide Remot (Bullock *et al*., 1983) have been reported to be effective in controlling EMR. However, no one these antibacterial drugs are registered with the USA Food and Drug Administration for control of ERM in cultured food fish. Florfenicol is used in aquaculture to control susceptible bacterial diseases (Fukui *et al*., 1987; Inglis *et al*., 1991). It has been demonstrated that florfenicol is effective in the treatment of enteric redmoth disease (Schering-Plough Animal Health, Aquaculture. Disease Management, Salmon- ERM).

In this study, it was used from florfenicol to control mortality due to ERM disease. Evaluation of efficacy florfenicol was based on mortality with dead fish collected daily and the percentage mortality calculated for the 3 days after withdrawal medication in which mortality ceases. The majority of deaths due to ERM disease occurred during the first day of medication (8.46%). Cumulative losses decreased from the 2nd day of medication and reached 8.08%. This value was 0.82% at the end of period treatment and reached 0.09% at the end of the third days of withdrawal drugs (Fig.6). Overall mortality was 1,934 including 522 for FF group, 558 for OTC group, and 854 for unmedicated group at the end of duration experiments (Fig.7). Cumulative losses were 36.50% (29.79% for FF, 30.00% for OTC and 49.73% for unmedicated group) at the end of duration experiments (Fig.8). Overall specific mortality rates were 0.10% (range 0.00 to 0.05) for FF, 0.68% (range 0.07 to 0.76) for OTC, and 1.50% (range 0.23 to 1.91) for unmedicated group at the end of third days of withdrawal drugs period.

Florfenicol at a dose rate of 10mg/kg body weight for 10 consecutive days resulted in a rapid decline in mortality rate caused by ERM disease after period treatment. These results were shown that florfenicol reduced mortality rate more than oxytetracycline (6.8 as much), and the unmedicated group (15 as much) at the end of third days after period treatment. It seems that the FF has preference over the OTC in treatment of ERM disease. Both FF and OTC were well tolerated by the fish. Mortality levels of 15–20% have been reported even with antibiotic treatment. In this study, ERM disease was successfully controlled by a combination of antibacterial therapy and environmental improvement in the affected farm.

Figure - 1 shows 2500 liter round concrete pond (2 meter in diameter, 1.5 meter in height)

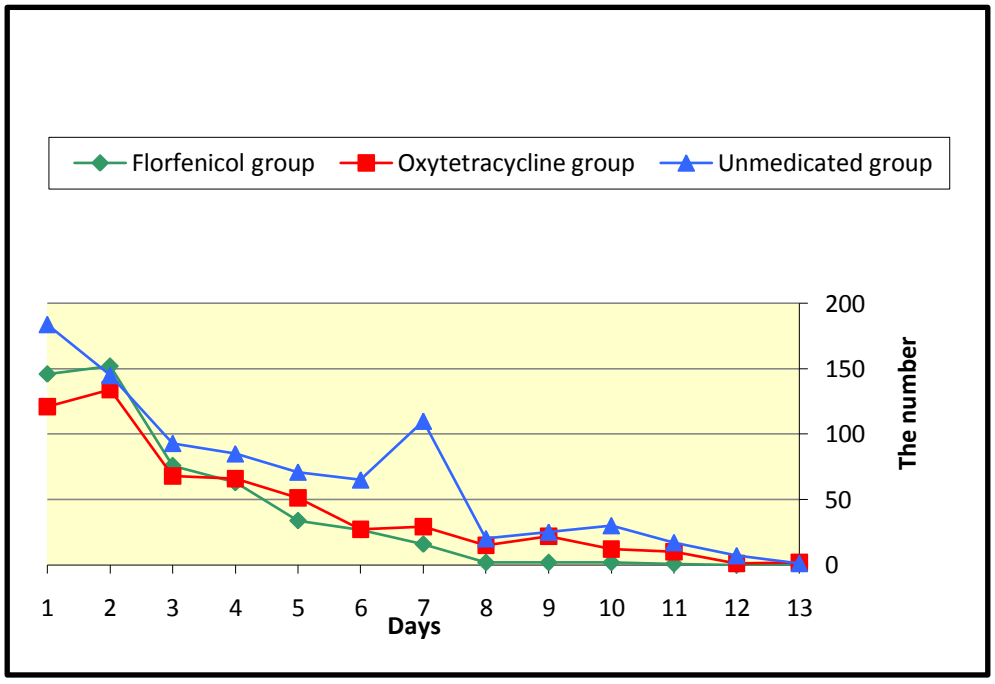


Figure – 2: Distribution of the number of losses by day in rainbow trout fry with ERM disease in separate ponds

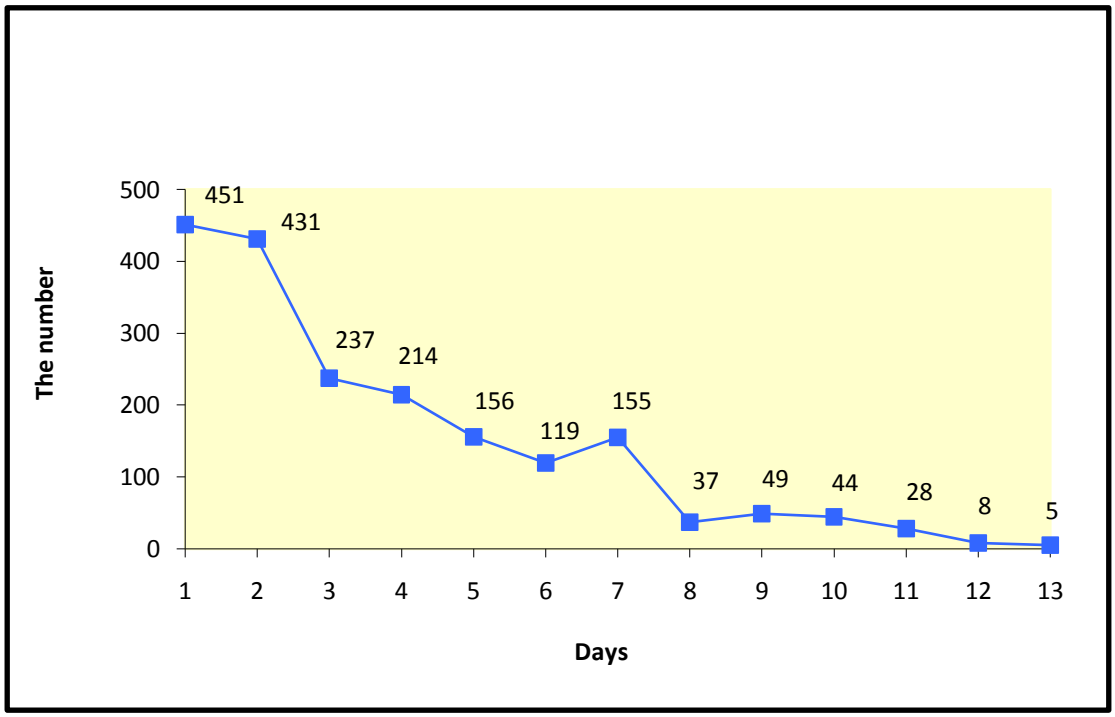
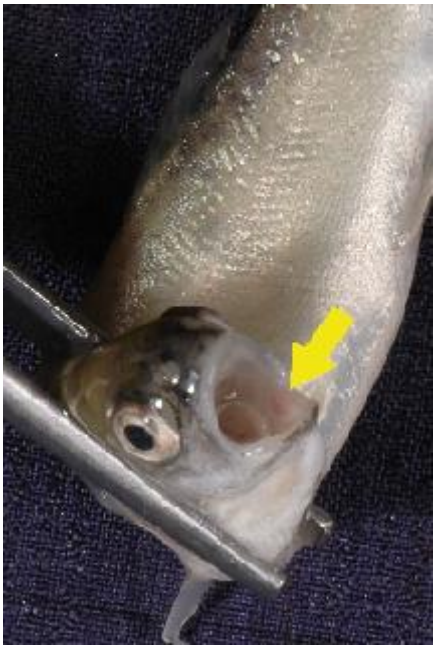


Figure – 3: The numbers of cumulative losses of rainbow trout fry with ERM disease in the three ponds daily



v

Figure – 4 shows small bright haemorrhage in oral cavity

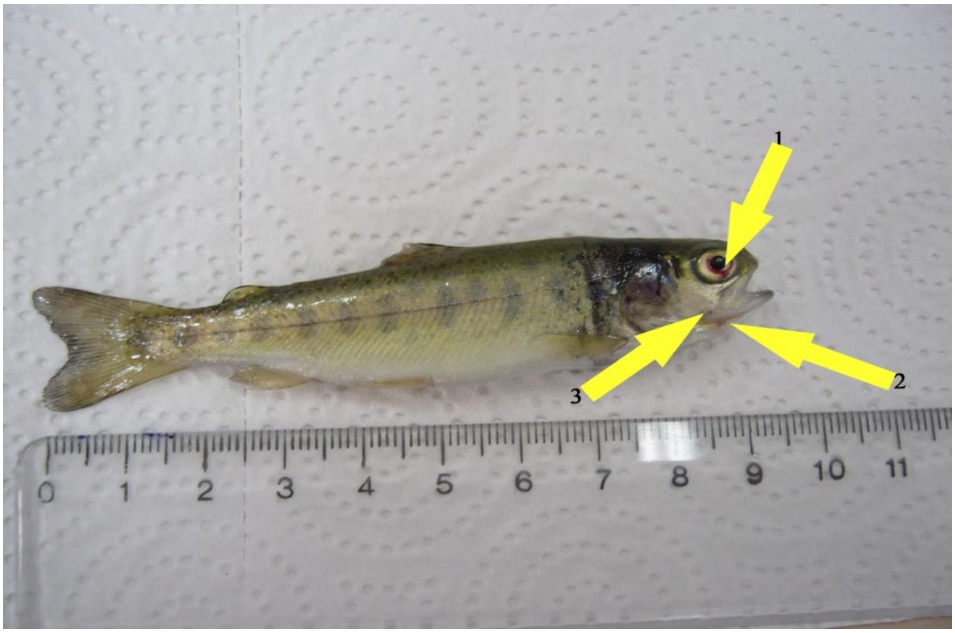


Figure - 5 shows the presence of haemorrhage on the around iris (arrow 1), petechial haemorrhages on the surface of the inferior mandible (arrows 2 and 3)

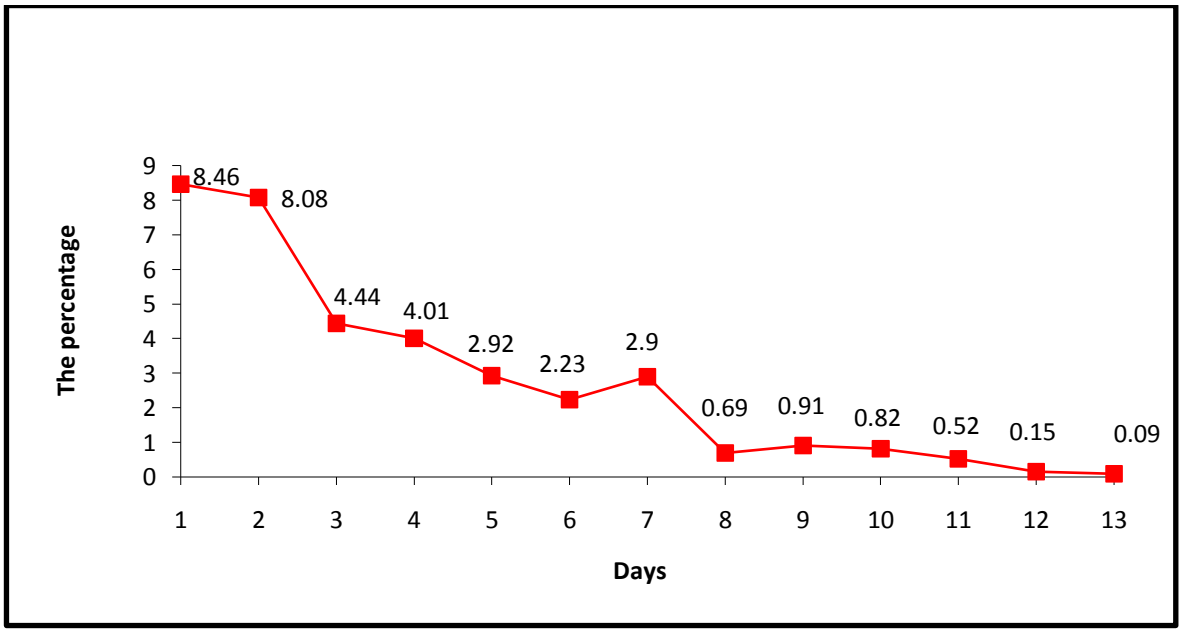


Figure – 6: The percentage of cumulative losses in rainbow trout fry with ERM disease in three ponds daily

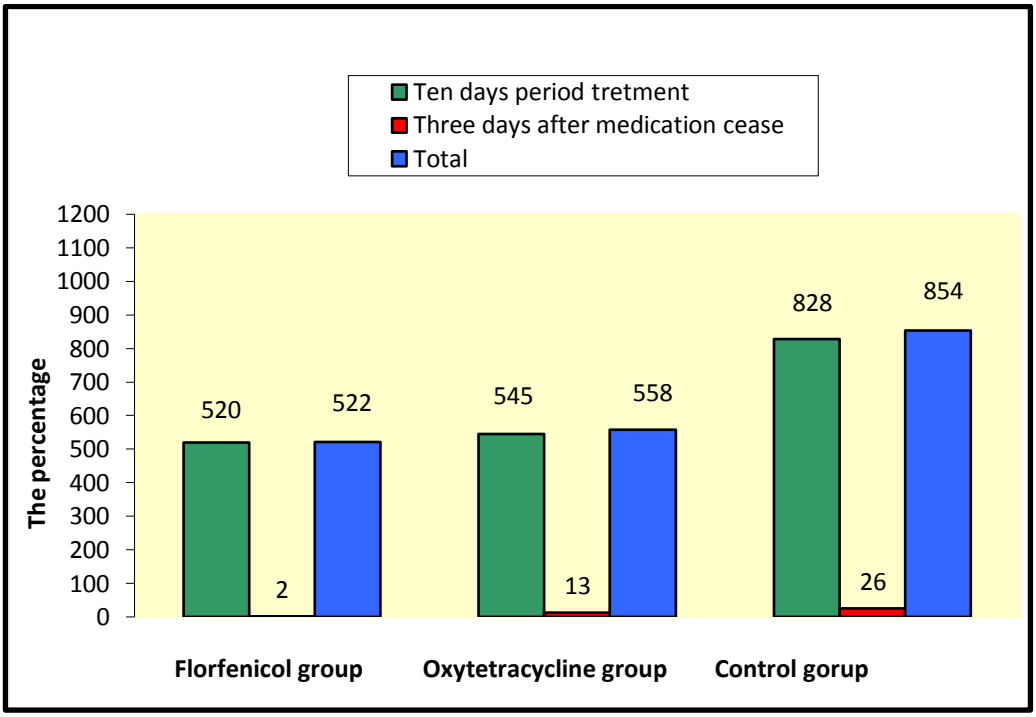


Figure – 7: The number of cumulative losses during and after medication in different groups

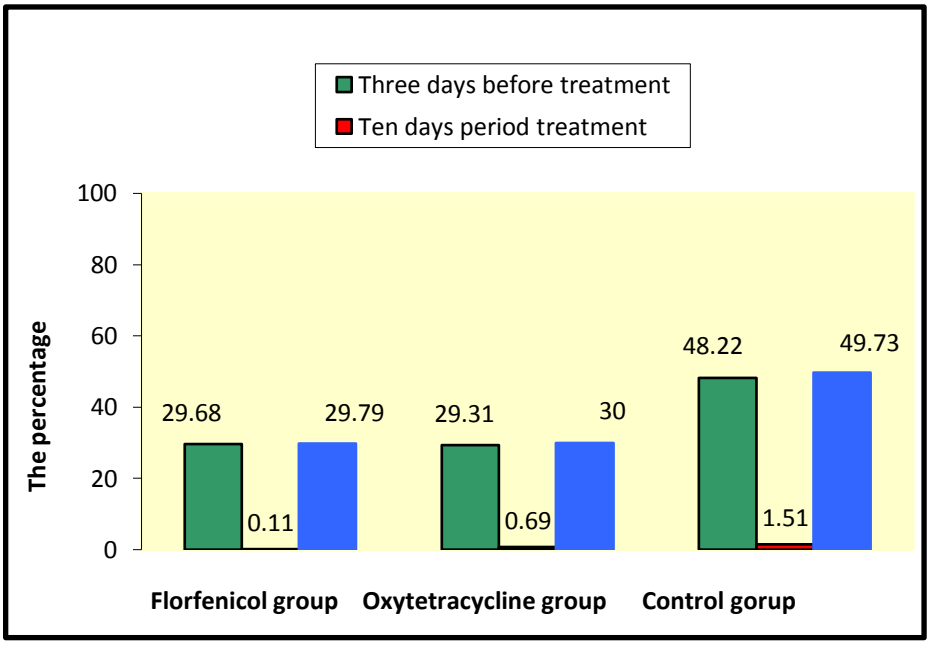


Figure – 8: The percentage cumulative losses rainbow tr

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