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Evaluation of Hepatic Function in Foals with Rhodococcosis after Treatment with Azithromycin and Rifampin

Ali Hassanpour[1*] and Hamidreza Alipour Kheirkhah[2]

[1]Department of Clinical Science, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
[2]Graduate of Veterinary Medicine, College of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

Authors' contributions

This work was carried out in collaboration between both authors. Author AH designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author HAK managed the literature searches, analyses of the study performed the spectroscopy analysis and author AH managed the experimental process. Both authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

This study was carried out to evaluate the effect of two antimicrobials, (*azithromycin* and *rifampin*), on Rhodococcosis, a foal disease caused by *Rhodococcus equi* and to evaluate potential effects of the treatment on foals' hepatic function. This study was conducted on 17 infected 2-15 months old foals, while 18 healthy foals were kept as control. *Rhodococcosis* in foals was confirmed by clinical signs and laboratory findings. In order to assess hepatic function the following enzymes were measured in serum of foals: Gama Glutamine Transferase (GGT), Aspartate Aminotransferase (AST), Alanin Aminotransferase (ALT), Alkalin Phosphatase (ALP) and Bilirubin (BIL). For

**Corresponding author: Email: alihassanpour53@gmail.com, A_hasanpour@iaut.ac.ir;*

treatment, *azithromycin* in combination with *rifampin* orally once a day for 2 weeks were used. At the end of the treatment, clinical symptoms resolved; however, it was observed intestinal disorders in the 13 foals. Among infected foals, serum markers of liver damage increased. Mean serum activity of GGT, AST and ALT was (P<.05) significantly higher among infected foals before treatment than among control foals, while the infected foals and mean difference between control and infected after treatment was not significant (P>.05). Serum activity of ALP in the diseased foal had a significant increase and mean difference between all three groups was significant (P<.05). Though mean serum levels of total BIL among sick foals was significantly (P<.05) lower than before treatment, it was significantly (P<.05) higher than control, and despite decreased, after treatment compared to before treatment, but not to normal and mean difference between 3 groups was significant (P<.05).

The findings of this study indicate that *azithromycin* in combination with *rifampin* was effective in the treatment of foals with bronchopneumonia by *R. equi*. Additionally, no adverse effects were noticeable on hepatic function.

Keywords: Equine; rhodococcosis; liver; antimicrobials; R. equi.

1. INTRODUCTION

Rhodococcosis is a microbial disease caused by *R. equi*. This bacterium is Gram- positive cocci and also causes bronchopneumonia, pneumonia, pleura pneumonia, polyarthritis and osteomyelitis in foals. In adult mare it may cause abortion. The disease is more prevalent among the 1-4 months old foals, and *R. equi* infection could became chronic. Clinical sign of bronchopneumonia include cough, anorexia, fever, depression, tachypnea, wheeze and crackle in lung auscultation, seldom nasal discharge [1]. Where the disease causes lung abscess, it is possible not hear abnormal sounds. In 20% of infected foals, it leads to non-painful polyarthritis. In 10% of foals, it leads to immune-mediated uveitis. Sometimes it may form abdominal cavity and other membrane abscess. Ultrasound and radiography are effective in diagnosis of lung abscess. In laboratory finding, there is leukocytosis and increased serum fibrinogen in infected foals, and while the amyloid A level in serum rises [2]. Conformation of the pulmonary disease which caused by rhodococcosis is based on culture of tracheal secretion and bronchial isolation obtained by aspiration of the trachea or alveolar lavage. In 86% of cases, culturing provides good result. Polymerase Chain Reaction (PCR) is another definitive diagnosis that is 100% successful [3]. This disease must be differentiating from interstitial pneumonia, viral, other bacterial pneumonia such as *Pasturella* spp. and *Streptococcus* spp. pneumonia, and parasitic pneumonia. It is possible that the functions of other organs such as the liver, heart, kidneys and gastrointestinal tract get affected. While the impact of this disease on the respiratory system is fully understood, the effect of *rhodococcosis* on other organs has not been comprehensively understood. This study was carried out to assess the impact of the disease on the functions of liver. Various approaches are used for the treatment of *R. equi*. The use of different antibiotics such as *penicillin* alone or in combined with *gentamicin, sulfadiazine* in combination with *trimethoprim, erythromycin* in combination *rifampin*, *clarithromycin* and *azithromycin* in combination with *rifampin* for treatment of this disease were proposed [4]. Among the mentioned methods of treatment the most effective therapy is *azithromycin* in combination with *rifampin,* which should be continued for as long as 2 weeks to a month until symptoms disappeared. Due to the long period of treatment, antimicrobials used may have adverse effects on the liver, heart, kidneys and digestive system of the animal [5].

Azithromycin is a macrolide antibiotic which is used to treat certain bacterial infections such as respiratory tract infections, genital tract, ear, skin, throat, and other infections. Side effects include diarrhea, abdominal pain, nausea and vomiting, mouth and tongue sores [5].

Rifampin or *rifampicin* is of the antibiotics from rifamycin group of which has the power to kill bacteria. It is a semi-synthetic compound derived from *Amycolatopsis rifamycinica*. Liver failure is the most important side effect of this drug and infected who receive this drug should always get liver function tests [6].

For evaluation of hepatic function, serum activity of Transferase *Gamma-glutamyl transferase* (GGT), *Aspartate Aminotransferase* (AST),

Alanin Aminotransferase (ALT) and *Alkalin Phosphatase* (ALP) enzymes are measured [7].

This study was carried out to evaluate the effect of two antimicrobials, (*azithromycin* and *rifampin*), on Rhodococcosis, a foal disease caused by *Rhodococcus equi* and to evaluate potential effects of the treatment on foals' hepatic function.

2. MATERIALS AND METHODS

This study was performed using 17 infected 2-15 months old foals in Tabriz area in Iran during 3 months (2014). Foals sickness was confirmed by clinical sign and laboratory findings. For the definitive laboratory confirmation, aspirated tracheal discharge was cultured in the microbiology and clinical pathology laboratory at the Veterinary Faculty of Islamic Azad University of Tabriz for the isolation of *R. equi* (soaping method used for taken tracheal and nasal discharges). 20 ml blood sample was collected from jugular vein of each diseased foal and after serum separated from each sample was frozen. For each sick foal general examination was done and the cardiac functional status was assessed clinically. Also to check the status of each animal's hepatic function, serum activity of GGT, AST, ALT, ALP and BIL enzymes was measured. Serum level of LDH, AST, GGT, ALT, ALP and Total Bilirubin were measured by using biochemical kits (All of the biochemical kits were *Zhist Shimi* (Iran)). Blood and serum samples thereof were obtained from each of the 18 healthy foals of the control group. In both sick and control groups averages were calculated and compared.

Each sick foal, was treated with 10 mg/kg of *azithromycin* (200 mg *azithromycin* human approved syrup) in combination with 10 mg/kg *rifampin* (300 mg human approved capsules) (dissolved in water). The drugs were administered orally once a day for 2 weeks [3]. At the end of the treatment, the clinical condition of the animals examined. And if problem persist, supplying drug was discontinued. Blood samples were collected after treatment, and data from the measurements were compared with corresponding pre-treatment data.

2.1 Statistical Analysis

The population was 20 foals infected with *R. equi* and 20 healthy foals. The sample size of 20 foals for each group was determined based on $n = \frac{z^2(1-\frac{\alpha}{2}) \times \sigma^2}{d^2}$ formula.

The results of the study were analysed using the Analysis of Variance (ANOVA) of the Statistical Package for the Social Sciences (SPSS) software.

3. RESULTS

In all 17 infected foals, clinical signs were fever, dry and seldom wet cough, nasal discharge, anorexia and asthenia. After treatment diseased foals, they had partial appetite, fever and cough disappeared, but 13 foals had loose feces and 4 foals suffered severe diarrhea. These 4 foals were treated with oral *sulfadimidin* and rehydration after testing the blood samples (Table 1).

In order to evaluate the hepatic function of the animals, hepatic enzymes were measured in the serum of the foals, allocated in three groups: control foals, foals infected before treatment and after treatment with antimicrobials. Mean serum activity of GGT, AST and ALT in *R. equi* infected group before treatment was significantly (P<.05) higher than in control group, and the mean difference between control and *R. equi* infected group after treatment was not significant (P>.05). Serum activity of ALP in *R. equi* infected group was significantly (P<.05) increased, while the mean difference of this enzyme between all three groups was significant (P<.05). Though mean serum levels of Total Bilirubin were significantly (P<.05) lower among foals after treatment, these bilirubin levels were still significantly (P<.05) higher than among foals in the control group (Table 2).

4. DISCUSSION

Rhodococcosis is a microbial disease caused by *R. equi*. This bacterium causes bronchopneumonia with clinical signs of cough, anorexia, fever, depression, tachypnea, wheeze and crackle in lung auscultation, seldom nasal discharge [1]. In foals where the disease results in lung abscesses, it is possible not to hear abnormal sounds [2]. In this study all 17 diseased foals showed clinical signs of fever, dry cough and sometimes wet, nasal discharge, anorexia, asthenia. After treatment all signs disappeared and it was found that using

Table 1. Clinical findings in foals with *R. equi* infection before and after treatment*

Clinical finding / Time of examination	Fever	Cough	Nasal ischarge	Anorexia	Lethargy and fatigue	Diarrhea	
						Pasty	Watery
Before Treatment	100% (17/17)	100% (17/17)	88.23% (15/17)	100% (17/17)	82.35% (14/17)	5.88% (1/17)	0% (0/17)
After Treatment	0% (0/17)	11.76% (2/17)	0% (0/17)	17.64% (3/17)	0% (0/17)	52.94% (9/17)	23/52% (4/14)

**Combination of both antimicrobials azithromycin with rifampin (10mg each antimicrobial/kg) administered orally once a day for 2 weeks*

Table 2. Comparison of serum markers of hepatic function in *R.equi* infected foals before and after treatment with the control group

Group	GGT(U/L)	ALP(U/L)	ALT(U/L)	AST(U/L)	BIL(mg/dl)
Control	26.32±2.85 [a]	485.24±12.87 [a]	13.87±2.22 [a]	329.40±11.42 [a]	328.39±12.27 [a]
Infected before treatment	37.14±4.12 [b]	679.73±11.81 [b]	14.04±1.27 [b]	394.29±17.58 [b]	412.24±9.81 [b]
Infected after treatment	28.74±5.31 [a]	532.65±16.56 [c]	12.9±3.32 [a]	336.52±20.14 [a]	386.3±10.46 [c]

Gama Glutamine Transferase (GGT), Alkalin Phosphatase (ALP), Alanin Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Bilirubin (BIL) enzymes

a, b, c: Different letters in each column indicate statistically significant differences (P=.05)

azithromycin in combination with *rifampin* was very useful for treatment of this disease. But 13 foals had loose feces which was severe diarrhea. After blood tests, the four foals were treated with oral *sulfadimidin* and rehydration. Occurrence of diarrhea in these infected foals could be due to prolonged use of *azithromycin* and *rifampin. Azithromycin* is a macrolide antibiotic which is used to treat certain bacterial infections such as respiratory tract infections, genital tract, ear, skin, throat, and other infections. Side effects include diarrhea, abdominal pain, nausea and vomiting, mouth and tongue sores [5].

Rifampin or *rifampicin* belongs to the rifamycin group of antibiotic which has the power to kill bacteria. It is a semi-synthetic compound derived from *A. rifamycinica*. Hepatic failure is the most important side effect of this drug and diseased, who receive this drug should always get liver function tests [6].

Though mean serum activity of GGT, AST and ALT were significantly (P<.05) lower in the sick foals after treatment, levels of these enzymes were still significantly (P<.05) higher than were found in foals of the control group. Treatment with mentioned drugs decreased serum level of these enzymes and mean difference between control and infected group after treatment was not significant. Though serum activity of ALP and BIL decreased in foals after treatment, levels of these enzymes were still significantly (P<.05) higher than were found in foals of the control group. It was found that treating diseased foals with *azithromycin* in combination with *rifampin* improved serum activity of AST, GGT and ALT but did not affect ALP and bilirubin. This finding is different from another report that prolonged use of *rifampin* could cause hepatic failure [6], changes in serum activity of ALP could be due to damaged tissues such as lung, muscle, and liver.

Hyperbilirubinemia occurs when bilirubin levels go higher than 1 mg/dL. This problem may be due to the amount of excreted bilirubin which is higher than capacity of the liver. Obstruction of bile duct could cause by hyperbilirubinemia. Excess bilirubin complex in the blood could cause jaundice [3]. In a study in Australia on 22 horses which were experimentally poisoned with pyrrolizidine alkaloids, serum bilirubin increased [8]. In horses that suffer from cholestatic liver, increasing serum total bilirubin has been reported [9].

ALP is an enzyme mostly produced in liver and bone marrow. Also this enzyme can be extracted from intestine, kidney and placenta. ALP test is useful for diagnosing liver and bone diseases. In cases of mild hepatocellular injury, there may be only mildly elevated levels of ALP. But in severe liver disease, it can substantially increase. Upon transition from acute phase, serum level

suddenly decreases, while serum bilirubin remains high [3]. Though measurement of AST in hepatic disease of Large Animal due to minor changes of ALT is useful, AST is non-specific for liver and increased AST in most tissue cell necrosis, including the cardia and skeletal muscle, and liver parenchymaplaced could be seen. So in evaluating this enzyme, we should ascertain the health of other organ [3]. On the other hand, it is believed that bilirubin and AST are sensitive indicators of liver function in cows near calving periods [10]. Lipopolysaccharide's (LPS) clearance in liver coopfer cells can lead to an increase in AST which is an unspecific hepatic enzyme [11]. AST can be found in the cytoplasm and mitochondria of such tissue cells with high metabolism line in heart muscle and liver, and at low levels in kidney, pancreas and brain. When disease or injury affects these cells, these cells get lysed. Released AST is removed by blood and its serum level increases. AST increase is directly related to the number of damaged cells. The level of increase depends on the time interval between injury and bleeding. This increase is observable 8 hours after cell damage. Within 24-36 hours, it reaches its maximum and within 3-7 days it returns to normal. If the cellular injury is chronic, these enzyme levels will be consistently high. For example, the serum concentration increases during heart attacks and muscle problems [3]. Lactate dehydrogenase is a non-specific enzyme found mainly in muscles, liver and blood cells. For this reason, the measurement of this enzyme activity in liver diseases in veterinary medicine is not recommended [12].

GGT is an enzyme that is mostly used for evaluating bile duct health. GGT is an enzyme that could be found in most organs such as liver, kidney, spleen and pancreas. Since the main source of GGT is the liver, in GGT any hepatic changes are more sensitive than changes in ALP. GGT level in the body is very limited, but when the liver is injured (due to obstruction of the bile duct because of tumors, stones or any other reason) the first liver enzyme that increases in the blood is GGT. This test helps to differentiate hepatic diseases from each other, but when there is an accompanying with rise in alkaline phosphatase, GGT is useful for distinguishing bone and hepatic diseases from each other. This enzyme increases in hepatic disease but does not change in bone disease, while ALP increases in both. In order to determine the cause of the increase in ALP, GGT levels should be measured [3].

In a study on horses that suffered experimental poisoning with pyrrolizidine alkaloids, resulting in hepatic injury, serum level of GGT, ALT, ALP, AST, BIL, Albumin and Total Protein. In these tests, measure sensitivity of GGT in detecting hepatic damage was 75% and specificity was 90%, while ALP measurement showed a sensitivity of 58% [8].

Foals in first 12 hours of their life had high level of ALP, GGT, LPH, cholesterol, triglycerides and BIL in serum but these levels decreased. In foals with hepatic disease (congenital jaundice) these parameters were steadily increasing [13]. In horses with hepatic cholestasis, serum bilirubin, ALP, GGT were high, while GGT and ALP served as indicator of hepatic damage [9]. On the other hand, increased GGT has been reported in horses suffering from amyloidosis [14].

The findings of this study indicate that *azithromycin* in combination with *rifampin* was effective in the treatment of foals, with *bronchopneumonia* caused by *R. equi;* however, no adverse effect was detected on hepatic function.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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