EFFECT ON BIOCHEMICAL PARAMETERS IN NATURALLY INFECTED CALVES WITH GIARDIASIS AFTER TREATMENT WITH ALBENDAZOLE IN VAN PROVINCE, TURKEY

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ABSTRACT

The aim of this study was to investigate the changes in the blood serum levels of some biochemical parameters, lipid-soluble vitamins and trace elements in the calves naturally infected with Giardia spp. in Van region, before and after treatment with orally 20 mg/kg albendazole three successive days. Serum samples were analyzed for biochemical parameters and some vitamins. In the infected animals, concentrations of creatinine, amylase levels were lower (p< 0.05). Glucose, urea, total protein, AST, ALT, sodium, potassium, retinol and α-tocopherol levels were seen significantly higher in the after treatment group (p< 0.05). There was no statistically significant difference in the concentration of total bilirubin, chloride and vitamin D₃ between groups. All the animals were not encountered cysts or trophozoites of Giardia at the end of the treatment. The results of this study would suggest albendazole to be 100 % effective against giardiasis in all of the calves in Van region.

Key words: Albendazole, Biochemical parameters, Calves, Giardia.

INTRODUCTION

Giardia spp. is the most common and important gastrointestinal parasite and a frequent cause of diarrheal illness throughout the world and Van, Turkey (Hamnes et al., 2006; Gul et al. 2008). Giardia spp. is localized and multiplied in the small intestines of humans, domestic animals and wild animals (Thompson et al., 1993).

Definitive determination of pathogenesis is difficult because giardiasis is an asymptomatic and non-invasive disease in clinically. Symptomatic giardiosis should be considered in the differential diagnosis of many diarrheal syndromes. Clinical features are associated with infection range from acute, short-lasting diarrhea to chronic syndromes such as metabolic disorders, weight loss and retardation of growth especially in calves (Mebus et al., 1973; Xiao, 1994).

Pathophysiological changes include malabsorption and maldigestion of electrolytes, nutrients, and intestinal microvillus alterations. Giardiasis causes pathological changes in characterization with intestinal villous atrophy, ulceration loss of epithelial barrier function, increased permeability and narrowing of the lumen (Buret, 2007). Giardiasis is diagnosed by the detection of cysts or trophozoites in the feces. Giardia trophozoites are holding on too tight with absorbent discs the small intestine of calves. In calves, intermittent loss of cysts may continue for several weeks (Xiao and Herd, 1994). In general gastrointestinal parasites reduce nutrient availability to the host through both reductions in voluntary feed intake and reductions in the efficiency of absorbed nutrients and lead to disorders of protein metabolism and fluid balance (Coskun, 1989). Prolonged malabsorption of fat and its excretion in stools can lead to a significant loss of A, D, E, K (Vitamins) & iron, especially as a result of chronic infection. Immunodeficiency with varying degrees of hypogammaglobulinemia orgammaglobulinemia predisposes to the acquisition of giardiasis and is

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the most commonly reported form of immunodeficiency associated with chronic giardiasis (Farthing, 1996; Koski and Scott, 2001). Giardiasis affect the intestinal absorption of macro and micro elements in directly or indirectly (Hesham et al., 2004).

Albendazole probably has the widest range of uses of all the benzimidazole carbamate with anithelmintic and antiprotozoal activity against intestinal and tissue parasites. Especially, albendazole was found to be very effective on giardiosis. The mechanism of action of albendazole, after being metabolized in the liver into albendazole sulfoxide, is to inhibit tubulin polymerization and the enzyme fumarate reductase and subsequently decrease glucose uptake and depletes their glycogen stores and parasites die in hunger of glycogen (Ayaz et al., 2010; Stukelj et al., 2011).

The aim of this study was to investigate the changes in the sero-biochemical parameters, lipid-soluble vitamin levels and trace elements in the calves naturally infected with Giardia spp. in Van region before and after treatment with albendazole.

MATERIALS AND METHODS

Animals: The materials used in this study were obtained from a farm in Van Province, in the east of Turkey. A total of 20 calves aged 1-3 months old, average body weight of 50-70 kg with diarrhea in 20 calves dehydration and naturally infected with Giardia spp. were used. Infected animals were treated with orally 20 mg/kg albendazole (Albecure, TEKNOVET) 3 consecutive days. Fecal samples were obtained before and after the treatment of this study directly from the rectum of each calf using sterile disposable plastic gloves to count cysts in feces. The fecal samples were transported to the laboratory in a labelled cool box and then stored for a maximum of 24 h before analysis. Blood samples were collected from infected calves before treatment and 18 days of treatment. Because of investigated the efficacy and tolerance of albendazol in the treatment of giardiasis in calves after the start of treatment but significance is from the second week (Albay et al., 2011; Lihua et al., 1996).

Parasitic examination: Fecal samples were obtained directly from rectum of each animal before and after the treatment of albendazole; consecutive 3 days dose of 20 mg/kg immediately placed in uniquely labeled specimen containers and stored on frozen cold packs until transported to the laboratory. Fresh stool specimens in previously distributed small plastic containers were collected from calves and immediately transported to the Parasitology laboratory of the Medicine Faculty in Yuzuncu Yil University. Macroscopically the stool samples were usually offensive, bulky, pale, non-bloody, mucoid (fatty) or watery. The stool samples were examined by native-Lugol and flotation (in saturated zinc sulphate) methods before treatment and after the treatment during 3 consecutive days. The numbers of cysts per gram of feces were counted by using the formula adopted by Handley et al. before treatment and after the treatment day of 18 (O’Handley et al., 1999).

Biochemical analysis: Blood samples were obtained from the jugular vein of calves from before the albendazole treatment and after the treatment day of 18 with orally albendazole at consecutive 3 days dose of 20 mg/kg. Serum samples were separated and analyzed for biochemical parameters (Sodium, potassium, calcium, total protein, albumin, glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by autoanalyser (Modular PP, Roche/Hitachi, Japan) and some vitamins (retinol, \( \alpha \)-tocopherol and Vit D,3) by high performance liquid chromatography (HPLC, Agilent-1100, Germany) method (Miller and Yang, 1985; Zaspel and Csallany, 1983; Reynolds and Judd, 1984). Measurements for levels of serum mineral elements were carried out by Atomic Absorption Spectrophotometer (UNICAM 929) (Ozdal et al, 2009).

Statistical analysis: Statistical analysis was performed using the SPSS 11,5 statistical program values were expressed as mean standard error. The results obtained were evaluated by paired samples t-test. The significant level was set at p<0.05.

RESULTS AND DISCUSSION

The data obtained are summarized in Tables 1 and 2. The consecutive 3 day albendazole treatment was found to be hundred per cent effective against giardiasis in all the calves. Animals were monitored for side effects observed for 30 days after the treatment. During and after the treatment of
After Treatment, 1995; 0± 0** 

The giardiasis could persist for several months and results in numerous episodes of diarrhea (Regassa et al., 1996). The economic losses caused by the flagellated protozoan is an important intestinal parasite.

Giardia spp. in calves causes regional economic losses in the world and in Turkey (El-Ghari et al., 1994; Galiero et al., 1994; Olson et al., 2004). The economic losses caused by gastrointestinal parasites are multifarious: lowered fertility, reduced work capacity, involuntary culling, and reduction in food efficacy, lethargy and poor condition. Parasitised animals gain lower weight, lower milk production and treatment cost and heavy mortality (Regassa et al., 2006). In this study, inappetence, weakness, diarrhea, dehydration, weight loss were observed in calves infected with giardiosis.

The giardiasis could persist for several months and results in numerous episodes of diarrhea (Xiao and Herd, 1994). The specific mechanisms of Giardia spp. leading to diarrhea and intestinal malabsorption (Buret, 2007). Giardia infection could also lead to a lot of enzyme deficiencies such as lactase and disaccharidase deficiency in the microvillus. Due to lack of absorption in Giardiasis, it was expected to fall in levels of electrolytes. Such as, gastrointestinal parasitism can cause similar electrolyte disturbances classically associated with characterized by hyponatremia and hyperkalemia (DiBartola et al., 1985; Willard et al., 1991). As electrolytes, sodium(Na) and potassium (K) play a role acid-base balance and osmotic pressure. A decrease in these ions may lead to severe complications in body functions. Lack of macro-elements cause clinical disorder, yield loss and death in animals with parasites found sodium and potassium levels in animals with parasites lower than healthy animals (Tanyuksel et al., 1995; Krajnicakova et al., 2003; Kulcu and Yur, 2003). But Ayaz et al., (2007) reported that K and Na levels did not change significantly in animals infected with many endoparasites. Indeed, in this study, potassium and sodium levels were significantly higher in the after treatment group. Recent years in studies of Giardia infections detected that chloride ions were increased, even if chloride levels increased in this study but not statistically significant(Troeger et al., 2007).

Pharmacologically, there have been relatively few agents used in therapy. Benzimidazoles have been popular drugs for the treatment of giardiasis in calves (O’Handley et al., 1999; Ayaz et al., 2010). Albendazole inhibits polymerization of tubulin in the parasite and blocks the glucose uptake. It also interferes with the enzyme fumarate reductase and thus the energy level of the parasite diminish and death results (Goodman and Gilman, 1975). The uses of chemotherapeutic agents were highly effective, for the control of giardiasis in calves provided the opportunity to enhance the performance, to reduce clinical signs and to prevent environmental contamination (Karabay et al., 2004; Geurden et al., 2006). A lot of data suggest that albendazole can influence the therapy of human, canine, cattle, lamb, pig and mice giardiasis (Reynoldson et al., 1991; Xiao and Herd, 1994; Xiao, 1994;

### TABLE 1: Sero-biochemical parameters in calves naturally infected with giardiasis after treatment with albendazole.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before Treatment</th>
<th>After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>85.50± 8.50</td>
<td>109.50± 3.11*</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>12.67± 1.15</td>
<td>8.83± 1.11*</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.06± 0.10</td>
<td>0.74± 0.02**</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>6.43± 0.14</td>
<td>7.31± 0.16**</td>
</tr>
<tr>
<td>Total bilirubin (g/dL)</td>
<td>0.41±0.074</td>
<td>0.35±0.01</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>40.20± 2.11</td>
<td>63.40± 3.59**</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>7.80± 0.86</td>
<td>26.40± 2.69**</td>
</tr>
<tr>
<td>Amylase (IU/L)</td>
<td>34.55± 1.64</td>
<td>17.00± 2.32**</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>139.00± 1.34</td>
<td>144.17± 0.65**</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.67± 0.18</td>
<td>5.18± 0.11*</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>102.67± 1.12</td>
<td>96.50± 1.26</td>
</tr>
<tr>
<td>Retinol (µg/ml)</td>
<td>0.208± 0.008</td>
<td>0.31± 0.02*</td>
</tr>
<tr>
<td>á-tocopherol (µg/ml)</td>
<td>1.780± 0.106</td>
<td>2.31± 0.08**</td>
</tr>
<tr>
<td>Vitamin D3 (µg/ml)</td>
<td>0.02± 0.0047</td>
<td>0.02± 0.1004</td>
</tr>
</tbody>
</table>

*p< 0.05
** p< 0.01

### TABLE 2: Cysts count in per gram feces of calves with giardiasis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before Treatment</th>
<th>After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysts per gram feces (cpg)</td>
<td>1580± 80</td>
<td>0± 0**</td>
</tr>
</tbody>
</table>

*p< 0.05
**p< 0.011

albendazole, no adverse effects were observed in the calves.

In this study, inappetence, weakness, diarrhea, dehydration, weight loss was observed in calves infected with giardiosis. In calves with giardiasis were determined to have been reduced clinical signs by usage albendazole for the control of giardiasis.

Giardia the flagellated protozoan is an important intestinal parasite. Giardia spp. in calves causes regional economic losses in the world and in Turkey (El-Ghari et al., 1994; Galiero et al., 1994; Olson et al., 2004). The economic losses caused by gastrointestinal parasites are multifarious: lowered fertility, reduced work capacity, involuntary culling, and reduction in food efficacy, lethargy and poor condition. Parasitised animals gain lower weight, lower milk production and treatment cost and heavy mortality (Regassa et al., 2006). In this study, inappetence, weakness, diarrhea, dehydration, weight loss were observed in calves infected with giardiosis.
In animals infected with parasites exhibit important differences in biochemical blood parameters. Also, parasitic infestations cause severe health problems that may be accompanied by a decrease in some blood biochemical parameters, blood trace elements and mineral levels (Tanyuksel et al., 1995; Deger et al., 1997). Parasitic diseases such as giardiasis is considered by poor nutrition and lack of micronutrient, reduction of digestion and absorption of nutrients and chronic inflammation (Hesham et al., 2004). In this study glucose, urea, creatine, total protein, α-tocopherol, retinol, potassium, sodium, AST and ALT levels were significantly lower in the calves with giardiasis. In our study, the levels of glucose, total protein, urea, creatinine were low in before treatment group compared to after treatment group (p < 0.05). But, experimentally infected with giardia in dogs, total protein and urea levels did not find a difference between before treatment and after treatment group (Rosa et al., 2007). Total bilirubin did not differ between groups. A molecule of creatine plays a role as a store of energy in muscles and floats kidney glomerüles (Kaddurah-Daouk and Wyss, 2000). Used in the diagnosis of decreased insufficiency serum creatine, decreased total protein and urea levels used in the diagnosis of renal insufficiency and dehydration increase the level of serum creatine. We thought that as an indicator of dehydration of serum creatine and urea levels increased due to diarrhea in Giardiasis, and albendazole treatment decreased urea and creatine levels.

The differences in activities of some liver enzymes (AST, ALT, GGT) in serum is generally indicator of some pathological changes of tissue and liver. Decreased transaminase levels are commonly observed for substances that induced liver cytochrome P450. Down regulation of ALT synthesis would affect the ability of the liver to metabolize amino acids for energy production in the cell. In Bal1kc1 et al., (2005)’s study, ALT and AST levels were found significantly increases in dogs with diroflariasis (p < 0.05). But Rosa et al., (2007) reported that AST, urea and creatinine levels did not differ experimentally infected with Giardia in dogs. In our study, although ALT, AST and amylase activities showed a statistically significant change, ALT and AST activities in calves with infected Giardia spp. were higher than after treatment group calves (p< 0.05). Our observations were similar to those of Yuksek et al., (2006)’s study. In our study, the finding that liver enzymes did not increase significantly and albendazole treatment has no effect on liver. But amylase activities were lower than after treatment group calves (p< 0.05).

Plasma vitamin levels decreased in calves with Giardia sp. because of inappetence and especially decrease intake of foods. It has been demonstrated that plasma vitamin concentration was decreased in animals with protozoa and infection disease (Issi et al., 2001). Such as giardiosis, balance of antioxidant/prooxidan activity is disrupting (Delibas et al., 1996; Taskapan et al., 2007). Gastrointestinal tract parasites effected on the system of antioxidant/prooxidant balance that their destructive effects may cause lipid peroxidation and antioxidant substances that their protective effects have been decreased (Deger et al., 2001). Especially vitamin E and retinol are located in the antioxidants substance groups. In this study, in the before treatment group respectively retinol and α-tocopherol levels increased after treatment by albendazole. Our results supported to results of previous researches (Marinho et al., 1991). Plasma retinol levels decreased in children with giardiosis but it didn’t significantly (Chavalittamrong et al., 1980). However, a study in Tanzania has been reported that retinol deficiency encountered in patients with giardia parasitic infection, retinol levels were significantly increased in after treatment group (Sturchler et al., 1987). A study about effect of vitamin A/Zn supplementation on Ascaris and Giardia infected animals show that may impair intestinal absorption of retinol and that Zn along with vitamin A supplements may assist in repletion of vitamin A status (Marinho et al. 1991).

Compared to the control group, albendazole treatment reduced cpg counts by % 100 in all of the calves with Giardia sp. in Van region, Turkey. Animals were observed for side effects for 30 days after the treatment. During and after the treatment of albendazole, no adverse effects were observed in the calves. In Xiao et al., (1994)’s study albendazole was very effective against giardiosis in cattle. In Stukelj et al., (2011)’s study three weeks after the
albendazole therapy in pigs, Giardia was absent from the feces.

Development of resistance for benzimidazoles requires careful usage of these drugs worldwide (Upcroft et al., 1996; Schwab et al., 2005). Although albendazole is a novel drug, there are many forms in which resistance development are reported and local usage of albendazole is required carefully. No development of resistance has been come across in present study. These results indicate that the albendazole should be preferred for treatment of animals having Giardia sp. in calves in Van region, Turkey. This study albendazole was 100 percent effective against giardiosis. Animals were monitored for side effects observed for 18 days after the treatment. During and after the treatment of albendazole, no adverse effects were observed in the calves. Furthermore, no adverse effects were observed in the calves.

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