

# Efficacy of blood transfusion accompanied by antibiotics and B vitamins for the treatment of naturally occurring Leptospirosis in cattle

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## SUMMARY

In cattle with severe Leptospirosis, blood transfusion has not yet been evaluated as part of supportive treatment. The aim of this study was to determine the findings and to evaluate a whole blood transfusion as an adjunct to antibiotic and B vitamins in cattle with leptospirosis. Forty two cattle shedding urinary spirochetes and showing the clinical symptoms of Leptospirosis were randomly divided into two groups. L. Hardjo and L. Grippotyphosa were detected in 61.9% of serum samples by microscopic agglutination test. The cattle in group I (n=21) were administered dihydrostreptomycin-penicillin and vitamin B complex. The animals in group II (n=21) were given the same treatment, including a whole blood transfusion taken from healthy donors. Before the treatment, the mean values of erythrocytes, PCV, hemoglobin and platelets were low ( $P < 0.001$ ), while MCV ( $P < 0.05$ ) was high in both groups as compared to controls (n=10). The mean values of AST, ALT, ALP, GGT, BUN, creatinine and bilirubin were high ( $P < 0.001$ ) in both groups. Twelve cattle in group I (57.1%) and 19 cattle in group II (90.5%) were survived by the treatments ( $P < 0.05$ ). Thus, the administration of whole blood transfusion accompanied to antibiotics and vitamin B is rewarding in life-threatening cases.

**Keywords: Biochemistry, Blood Transfusion, Cattle, Hematology, Leptospirosis.**

## RÉSUMÉ

### Efficacité d'une transfusion sanguine associée à un traitement antibiotique et de la vitamine B dans le traitement de la leptospirose du bétail

La transfusion sanguine n'a pas été encore évaluée en tant qu'élément du traitement de support des cas graves de leptospirose du bétail. Le but de cette étude était d'évaluer l'intérêt d'une transfusion sanguine comme traitement adjuvant à l'antibiothérapie et à l'administration de vitamine B. Les animaux des groupes I et II excrétaient des spirochètes dans l'urine et présentaient des signes cliniques. Les animaux du groupe I (n=21) ont reçu un traitement associant dihydrostreptomycine-pénicilline et vitamine B. Les animaux du groupe II (n=21) ont reçu le même traitement, plus une transfusion sanguine entière prise sur des donneurs en bonne santé. Avant le traitement, les valeurs moyennes des érythrocytes, PCV, hémoglobine et plaquettes ont diminué ( $P < 0.001$ ), tandis que MCV ( $P < 0.05$ ) augmentait dans les deux groupes par rapport aux contrôles non malades (n=10). Les valeurs moyennes d'AST, d'ALT, d'ALP, d'GGT, d'BUN, de créatinine et de bilirubine ont augmenté ( $P < 0.001$ ) dans les deux groupes. De plus, L. Hardjo et L. Grippotyphosa ont été détectés dans 61.9 % d'échantillons de sérum par MAT. 12 animaux de groupe I (57.1 %) et 19 du groupe II (90.5 %) ont survécu ( $P < 0.05$ ). Ainsi, l'utilisation de la transfusion sanguine en soutien des antibiotiques et de la vitamine B améliore le traitement des cas sévères de leptospirose du bétail.

**Mots clés : Biochimie, Transfusion Sanguine, Bétail, Hématologie, Leptospirose.**

## Introduction

Leptospirosis is an infectious disease of ubiquitous distribution caused by pathogenic leptospirae. It has a wide spectrum of clinical manifestations, varying from mild anicteric infection to severe hemolytic syndrome. In cattle, acute hemolytic syndrome of leptospirosis has been reported characterized by fever, icterus, anemia and hemoglobinuria [3,13]. Without effective treatment, hemolytic syndrome in cattle may result in death. A high mortality rate of severe disease was determined to be associated with certain serotypes of leptospirae [17]. Serovar hardjo, pomona, and grippotyphosa have commonly been detected in cattle and various epidemiological presence and mortality rates worldwide have been determined [17,11]. Serological studies have been extensively carried out and future investigations are warranted in this area.

The pathogenesis and treatment of hemolytic leptospirosis in cattle has only been partly described. The severity of clinical symptoms depends on the degree of damage to multiple organs such as kidney, liver and lung, as well as hematological changes concerning hemolysis and endothelial impairment. The disease, for instance, causes a decrease in erythrocyte and platelet counts, leading to anemia and hemorrhagic diathesis, respectively. Elevated bilirubin levels result from hemolysis and hepato-renal failure, indicating the characteristic nature of clinical signs. Hemolytic anemia is due to hemolysins with phospholipases produced by the infecting leptospirae and is dependent upon the strains [18,19]. Leptospiral antigens and the damage caused are illustrated in the kidney and the liver [14]. The data obtained by hematology and biochemistry may reveal the severity of damage *in vivo* and may lead to an accurate treatment. Administration of whole blood transfusion has been reported as clinically very rewarding in cases of life-threatening anemia in cattle [16].

It is vital that donors and recipients be selected from the same herd to prevent transmission of latent infections between herds. Treatment should be initiated as early as possible and intensive care may be necessary in severe disease.

The purpose of this study was therefore to determine biochemical and hematological characteristics and to evaluate whole blood transfusion as an adjunct to antibiotic and vitamin B treatment in severe hemolytic leptospirosis of naturally infected cattle. Relevant aspects of the findings and management of severe leptospirosis were discussed as well.

## Materials and Methods

### ANIMALS AND SAMPLING

The animals used in this study were 42 cattle shedding spirochetes in urine and showing one of clinical symptoms of leptospirosis which were fever, anemia, icterus, and hemoglobinuria. Animals having clinical symptoms but not shedding spirochetes and/or any history of earlier infection or vaccination against leptospirosis were not included in the study. The cattle with leptospirosis were randomly divided into two groups and equal clinical severity between groups was relatively established, when admitted to clinic. Group I and II consisted of 21 diseased cattle each at the initiation of the treatments. Additionally, the control group contained 10 clinically healthy cattle. All clinical examinations were performed by the same researcher, Dr. Y. OZKANLAR. The mean ages and weights of the animals in group I, II and the control were  $2.8 \pm 1.2$ ,  $2.7 \pm 1.1$  and  $3.1 \pm 1.6$  years old, and  $275 \pm 61$ ,  $272 \pm 54$  and  $286 \pm 38$  kg, respectively. Animal breeds were holstein, brown swiss and anatolian mixed breed. Both clinically healthy donors and affected recipients were selected from the same herd to prevent possible transmission of infections between herds and to reduce blood group differences.

Urine samples were collected into sterile tubes. Blood samples were also collected into tubes – with EDTA and coagulation activator – from jugular veins. In group I and II, samples for biochemical and hematological analyses were collected just before the treatment and one day after the initiation of the treatment. Blood smears were also prepared for microscopic examination.

### SAMPLE ANALYSIS

A dark field microscopic examination was performed on centrifuged urine of all cattle. All serum samples were also analyzed by MAT in a reference laboratory (Etilik Veterinary Control and Research Institute in Ankara, Turkey) to detect the titers of leptospiral antibodies. MAT was performed using antigens against grippotyphosa, hardjo, pomona, canicola, hebdomadis and australis [13]. The titers of 1/200 and over agglutinations were assigned as positive results.

Hematological parameters of erythrocytes, packet cell volume (PCV), hemoglobin, platelets, mean corpuscular

volume (MCV) and leukocytes in the blood samples with EDTA were examined in the groups by a hematology analyzer (Beckman Coulter, USA). Biochemical parameters of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, and bilirubin were measured in the samples (microplate spectrophotometer, Bio-Tek uQuant, USA) using commercial test kits as instructed in the manufacturer's brochures (test kits, Immuno-Biological Laboratories, Inc., Germany). Biochemical analyses of urine were also conducted by chemstrips (Combur-Test, Roche Diagnostics, Germany). Additionally, any interference by possible protozoal agents (anaplasmosis, babesiosis, etc.) was excluded from the study by blood smear examinations.

### TREATMENT PROCEDURE

The treatment of group I consisted of 1 ml/20kg dihydrostreptomycin-penicillin (Reptopen S, Ceva-Dif, France – 200,000 IU benzylpenicillin procaine and 200 mg dihydrostreptomycin sulphate per 1 ml) i.m. once a day for 5 days and 1 ml/20kg vitamin B complex (Berovit B<sub>12</sub>, Ceva-Dif, Turkey – 5mg Vit B<sub>1</sub>, 2mg Vit B<sub>2</sub>, 2mg Vit B<sub>6</sub>, 4µg Vit B<sub>12</sub>, 20mg Niacin, 10mg D-pantothenol per 1 ml) i.m. once a day for 3 days.

The treatment procedure for group II was identical with group I (dihydrostreptomycin-penicillin and vitamin B complex), including a single whole blood transfusion on the first day of treatment. Whole blood of healthy adult donors was first collected in transfusion bags (Blood Pack Unit, USP, CPDA-1, Baxter Healthcare Corporation, USA) and then infused into each animal in group II (n=21) via jugular veins using a disposable blood transfusion set with cloth filter (Transfusion set, PL 70, Polymed Medical, Czech Republic). The total blood needed for each anemic animal was calculated individually using hematocrit (PCV) values to estimate the recipient's value to exceed 20% (i.e., an animal with 12% hematocrit value was given 1.5 L of whole blood per 100 kg of b.w., approximately) [12]. In most cases, one donor was used for one recipient. If more than 2.5 L of blood transfusion was needed for one recipient, two donors were used concerning donor's health. A lam agglutination test was applied to the serum of the recipient and the erythrocytes of the donor to verify compatibility (no agglutination) before the transfusion. All transfusions were performed on the same day of blood collection. Animals were monitored during transfusion for possible adverse reactions.

### STATISTICAL ANALYSIS

Biochemical and hematological parameters were compared in the groups. The data were analyzed by one way ANOVA using a computer-based statistics program (SigmaStat package software). Differences between groups are determined requiring a  $P < 0.05$  for significance. The values were presented as mean  $\pm$  standard error of the mean (SEM) in tables. Comparisons of survival and death numbers between two treatment groups were assessed by Chi Square

analysis. Survival rates and MAT results were also presented as percentages.

## Results

All animals in group I and II were showing clinical signs of hemolytic syndrome of Leptospirosis before the treatment. The clinical symptoms observed were fever, anemia, icterus, and hemoglobinuria including one or more additional findings of anorexia, apathy, dehydration, dyspnea, hyperpnea, bilirubinuria, mastitis and abortion in all affected cattle. Spirochetal leptospirae shedding was confirmed in all affected cattle in the group I and II by dark field microscopic examinations of urine. Additionally, 26 out of 42 serum samples (61.9%) were detected to be sero-positive against serovars of leptospirae. The titers in MAT in the groups are demonstrated in Table I.

Comparing the control, the values of erythrocytes, PCV, hemoglobin, and platelets were low ( $P<0.001$ ), while the value of MCV ( $P<0.05$ ) were high in group I and II before the treatment (Figure 1 A,B,C,D,E). Moderate increases in WBC values ( $P<0.05$ ) were present resulting from slight increases in neutrophil counts (Figure 1 F).

After the treatment, the values of erythrocytes and PCV levels were increased in group II ( $P<0.05$ ) (Figure 1 A,B);

hemoglobin and platelets tended to increase without having statistical significance (Figure 1 C,D); and MCV and leukocyte distributions were not changed (Figure 1 E,F). The activities of AST, ALT, ALP, and GGT and levels of BUN, creatinine, and total, direct and indirect bilirubin were incredibly high ( $P<0.001$ ) in group I and II compare to control (Table II). Urinary analyses revealed severe proteinuria, hemoglobinuria, hematuria, bilirubinuria and leukocyturia in group I and II before treatment.

It was obviously noticed that the severity of clinical symptoms in group II improved just after the blood transfusion. Gross hemoglobinuria disappeared on the third day, while the icterus disappeared on the fifth, approximately. Nineteen out of 21 cattle in group II (90.5%) survived through the administration of blood transfusion, antibiotics and B vitamins, while 12 out of 21 cattle in group I (57.1%) survived through the administration of antibiotic and B vitamins. One cattle died one day after admission to the clinic in group II and 4 cattle died one day after admission to the clinic and 5 cattle died later in group I. The number of survivals of observations in two treatment groups were significantly vary ( $P=0.035$ ) indicating the effectiveness of blood transfusion. No agglutination was observed in lam agglutination tests before transfusion. No serious adverse reactions to blood transfusions were noted except for a slight tremor in some animals.

Serogroup	Titers	Group I (n)	Group II (n)
Grippotyphosa	1/400	1	2
Grippotyphosa	1/800	3	5
Grippotyphosa	1/1600	3	1
Grippotyphosa	1/3200	1	1
Hardjo	1/200	1	-
Hardjo	1/400	-	2
Hardjo	1/800	2	1
Hardjo	1/1600	1	2

TABLE I: Titers against the serovars of leptospira and the numbers of animals in group I and II.

Parameters	Control	Group I	Group II
ALT (U/L)	23.0±3.1 <sup>a</sup>	98.7±12.4 <sup>b</sup>	100.9±14.7 <sup>b</sup>
AST (U/L)	84.1±11.6 <sup>a</sup>	300.6±25.6 <sup>b</sup>	314.8±26.7 <sup>b</sup>
GGT (U/L)	14.6±1.2 <sup>a</sup>	86.1±8 <sup>b</sup>	86.0±8.5 <sup>b</sup>
ALP (U/L)	44.4±10.8 <sup>a</sup>	921.4±151.5 <sup>b</sup>	938.8±148.7 <sup>b</sup>
BUN (mmol/L)	8.2±0.4 <sup>a</sup>	50.5±4.1 <sup>b</sup>	47.7±4.3 <sup>b</sup>
Creatinine (µmol/L)	116.0±3.9 <sup>a</sup>	267.4±16.9 <sup>b</sup>	263.4±17.4 <sup>b</sup>
TBIL (µmol/L)	8.6±1.6 <sup>a</sup>	140.6±16.4 <sup>b</sup>	143.6±17.6 <sup>b</sup>
DBIL (µmol/L)	3.4±0.8 <sup>a</sup>	120.9±15.5 <sup>b</sup>	119.5±18.5 <sup>b</sup>
IBIL (µmol/L)	5.1±0.7 <sup>a</sup>	36.7±5.7 <sup>b</sup>	37.4±6.8 <sup>b</sup>

TABLE II: Mean values of biochemical results of sera samples in control, groups I and II before treatment. Different letters as superscripts in the same column represent statistical difference ( $P<0.001$ ). ±: Standard error of the mean.

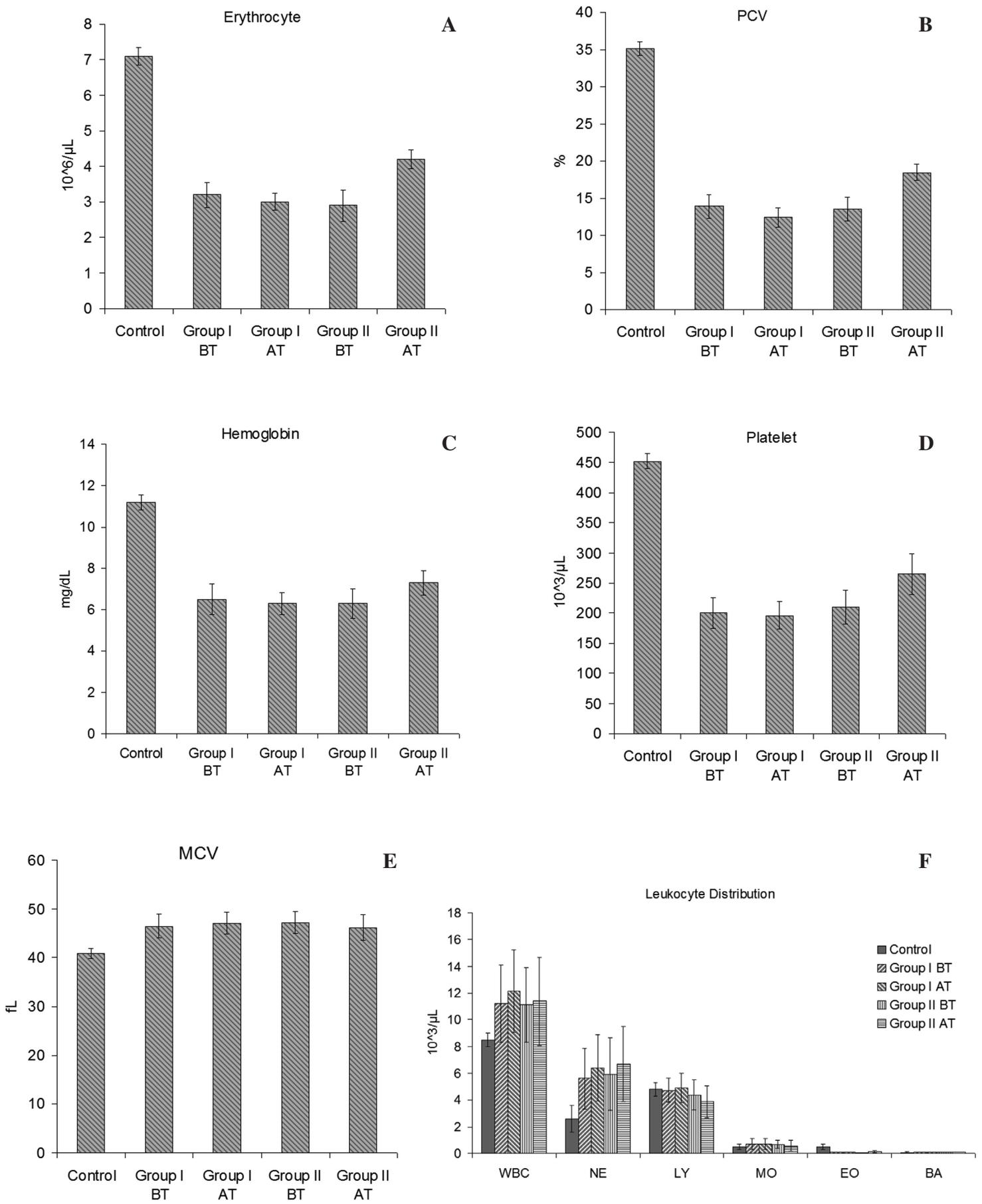


FIGURE 1 : Hematological parameters in control, group I and group II before and after the treatment. **A:** erythrocyt; **B:** PCV: Packet cell volume (Hematocrit), **C:** hemoglobin; **D:** platelet; **E:** MCV: Mean corpuscular volume, **F:** Leukocytes. BT: Before treatment. AT: After treatment. Bars represent standard error of the mean.

## Discussion

Leptospirosis has frequently been detected throughout the world, particularly in the Middle East, causing economic bankruptcies in herd production [11,13,15]. The mechanisms of leptospira-induced toxicity remain to be discovered, in part due to fragmentary findings. The findings of this study provide an attempt to reveal the complex pathogenesis of severe leptospirosis and address the timely transfusion of whole blood accompanied by traditional treatment of antibiotic and vitamin in naturally infected cattle.

Cattle showing clinical hemoglobinuria and/or icterus may be evaluated as having leptospirosis in endemic/epidemic regions. A dark field microscopic examination is a relatively easy and rapid test to confirm the presence of urinary spirochetes providing certain diagnosis. Regarding differential diagnosis, lymphadenopathy, which is not detected in the present cases, is generally present in protozoal diseases, and clostridial infections cause sudden death. In a recent study, 45% of 574 cattle showing the clinical signs were determined as sero-positive according to the results of MAT [13]. It was also reported that 1,254 cattle were found to be sero-positive in 15,596 samples, accounting for an 8.04% seroprevalence rate in Turkey, and the responsible serovars were grippotyphosa and hardjo. The serovars of hardjo, pomona, and grippotyphosa have also been identified in mature cattle in the USA [11] and Europe [15]. In the present study, grippotyphosa and hardjo serovars were detected, accounting for 61.9% in cattle showing bacteriuria and clinical signs. It is already known that, in sero-negative animals shedding spirochetes, an antibody response against the leptospirae might be seen within 10 days.

An acute hemolytic syndrome is frequently but not exclusively associated with infection due to serovars [18]. Hemolytic anemia due to hemolysins increases the mortality rate in cattle [3]. Hemoglobinemia can be observed in certain serovars of *Leptospira interrogans* related to serovar-host associations in calves [16]. It was also proposed that the erythrocyte lesions were due to the adverse effects of leptospiral toxin(s) upon the formation of defective portions of cytoplasm. This differs from the theory that the erythrocyte destruction was due to phospholipase-like toxins acting directly upon the erythrocyte membrane. In the present study, the serotypes of grippotyphosa and hardjo decreased the values of PCV, erythrocytes and hemoglobin severely. The leptospirae-toxins induce intravascular hemolysis [6], erythrocyte sequestration and erythrophagocytosis within the spleen, liver and bone marrow [17]. They also cause pore formation across the cell membrane and endothelial damage to the small blood vessels, leading to hemorrhage and necrosis in multiple organs [8]. Furthermore, platelet aggregation and Kupffer cell phagocytosis might potentially be the causes of thrombocytopenia in the disease [21]. Thrombocytopenia in the present cases may be responsible for aggravating hemolytic anemia through endothelial damage and hemorrhagic diathesis. During intravascular hemolysis and hemorrhagic diathesis, erythrocyte production is stimulated by the anemia, which results in an increased MCV value. However, a response to anemia can only be obtained when toxin-inflicted hemolysis and hemorrhage are stopped. In cattle with lep-

tospirosis, leukocytes are also raised in both groups compared to control ( $P < 0.05$ ) as an immune system defense mechanism against the bacteria, resulting in a moderate increase in neutrophil counts. Because leukocyturia has been present during the phase of spirocheturia, leukocytes in the present study could not be improved markedly in the cases after treatment.

Upon entering into the host, leptospirae spread immediately and are circulated within the bloodstream, although the organisms are phagocytosed by the macrophages. Leptospirae-induced damage to the capillary endothelia results in vasculitis, which is responsible for the most marked manifestations of the disease. The injured endothelia of small blood vessels, caused by the toxin(s), results in renal necrosis, hepatocellular damage, meningitis, myositis and placentitis [14]. Hypoxia, as a result of severe anemia, has a vital role in causing necrosis of multiple organs as well. Thrombocytopenia, dehydration, and altered capillary permeability may make contributions to the development of organ failure. The values of BUN and creatinine were obviously high in group I and II. These indicate the existence of renal failure, even though it is rather difficult to observe high levels of BUN because of the urea cycle in ruminants. High levels of AST, ALT, ALP and GGT showed destruction of hepatocytes and cholangiocytes, while the increased levels of bilirubin indicate both intravascular hemolysis and dysfunction of liver and kidneys. High GGT values might be typical of both liver and kidney diseases. Previous reports have determined related findings in cattle and lambs to the some extent [9,10]. Additionally, icterus, hepato-renal failure and thrombocytopenia can be seen in man with Weil's syndrome of leptospirosis [2,20]. Centrilobular necrosis in liver is also expected, although severe hepatocellular necrosis is not a common feature of leptospirosis.

The present data indicate that kidneys and liver were severely affected by leptospirosis, as discussed above. Large amount of endotoxins are released by the death of bacteria (typically spirochetes) faster than the body can remove the toxins via the natural detoxification processes performed by liver and kidneys. It is known that a Jarisch-Herxheimer reaction happens within hours following antimicrobial therapy in some human cases of leptospirosis [4,7]. Unfortunately, no study has evaluated whether this condition is present in cattle until now. The authors suggest that further studies should also be addressed to investigate Jarisch-Herxheimer reaction in cattle with leptospirosis, because antibiotics are always offered in the treatment of the disease.

Blood transfusion was reported to be quite effective in cases of life-threatening anemia in cattle [16]. Furthermore, the administration of dihydrostreptomycin-penicillin can eliminate the urinary shedding of spirochetes, although their mechanisms of effects in severe hemolytic leptospirosis have not been evaluated [1]. The present observations and previous reports suggest that timely transfusion of whole fresh blood be administered to overcome severe hemolytic leptospirosis. Indeed, transfusion – providing the vital components such as erythrocytes, platelets and plasma – contributed to repair the present collapses, i.e., anemia, hemorrhagic diathesis, septicemia and hepato-renal failure, in affected cattle

treated herein. A PCV value of 15% or less developing acutely may require transfusion, while chronic anemia can be tolerated in cattle without any transfusion [5,16]. Although the total amount of blood for each animal affected was given to ensure the recipient's PCV value would reach 20%, hematological analyses revealed lower levels. While 75% of bovine erythrocytes transfused are destroyed within 48 hours, several vital hours can be gained for natural erythropoietic response. The vitamin B complex administered may also contribute to improve erythropoiesis in both groups. The elevated PCV values in group II compared to group I indicated an obvious efficacy of the transfusion. Therefore, the number of survivors were significantly ( $P<0.05$ ) higher in group II (90.5%) than in group I (57.1%) indicating a certain efficacy of blood transfusion.

In conclusion, collapsed hematology, renal failure and hepatic destruction all deteriorate the clinical condition of the cattle affected. The combined administration of a whole blood transfusion accompanied by dihydrostreptomycin-penicillin and B vitamins was effective for treatment of life-threatening leptospirosis in the majority of cattle with leptospirosis.

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