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Evidence of Clinicopathological Changes during Equine Leptospirosis

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MSK, MA and MI designed the experiment. MYZ and MLS executed the experiment. MUS and ON analysed the data and wrote the manuscript.

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ABSTRACT

Leptospirosis is a globally spread zoonotic disease effecting a wide range of animal species and humans. 18 *Leptospira* positive horses based on ELISA and 9 healthy horses were included in the study. Blood profile, serum biochemistry and mineral profile were analyzed at day 0, 7 and 21 of infection. Results showed significant decrease ($P<0.05$) in red blood cells (RBC), packed cell volume (PCV), hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC) and platelets while total leucocytes count (TLC), neutrophils, eosinophils, basophils, lymphocytes, monocytes, red cell distribution width (RDW) and mean corpuscular volume (MCV) were increased significantly ($P < 0.05$) which indicated hemolytic anemia. While, serum biochemistry showed significant elevation in alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine which indicate renal and liver damage. Concentration of Na, K, Ca, Mg, P, Cu and zinc in serum were significantly decreased ($P< 0.05$), indicating tubular damage in kidneys.

INTRODUCTION

Leptospirosis is globally spread infectious disease which infects a variety of domestic and wild animals such as sheep, horse, cattle, dog, raccoon, pig, skunk, and even humans are not the exception (Kojouri *et al*., 2009; Saleem *et al*., 2013). It is caused by spirochetes *Leptospira* interrogans along with its more than 260 serovars (Tonin *et al*., 2012a). Prevalence of leptospiral serovars varies in a particular geographic region and for every serovar there exists one or more than one maintenance hosts which serve as the reservoir of infection (Balamurugan *et al*., 2013). It occurs sporadically throughout the year in these areas, with the highest incidence in warmer months (Tilahun *et al*., 2013), marked by heavy rainfall (Himani *et al*., 2013; Hamond *et al*., 2014). In milder cases horses may have fever, anorexia and listlessness but severe infection involves jaundice (mostly in foals, rarely in adult horses), anemia, conjuctival suffusions, pathechial hemorrhage on the mucosa and hematuria (Verma *et al*., 2013; Bauverud *et al*., 2009). Leptospirosis provokes clinical abnormalities that can lead to significant changes in hematological and other laboratory findings. Decreased erythrocyte count, low Hb concentration, PCV and lymphocytes are common lab findings (Tonin *et al*., 2012b). Leucocytosis, neutrophilia, eosinopenia, and lymphopenia, along with high serum bilirubin concentration have also been reported in equine leptospirosis (Pinna *et al*., 2013). Liver function tests revealed elevated levels of serum alanine aminotransferase (ALT), and aspartate aminotransferase (AST) (Tonin *et al*., 2012a). A comprehensive understanding of hematological, biochemical and mineral alterations is necessary to study the systemic effects and clinical pathology of leptospiral infection in horses, so present study was conducted to mark changes in clinicopathological parameters during equine leptospirosis.

MATERIAL AND METHODS

For the current study 18 leptospirosis ELISA positive horses were selected, along with 9 healthy horses (n=27). Routine feed and fresh water were provided *ad-libitum* during the experiment. Formal permission from the Ethical Review Committee for the Use of Laboratory Animals, University of Veterinary and Animal Sciences, Lahore were taken.

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Three ml blood was drawn from jugular venepuncture in each of plain (red top) and anticoagulant coated (purple top) vacutainers, by 20 gauge needle after disinfecting the collection site with alcohol swab. Blood collected in anticoagulant coated vacutainer was stored for hematological analysis and in plain vacutainers for biochemical and mineral analyses. Serum was immediately separated and refrigerated at -20°C till analyzed. Clinical pathological parameters including hematology, liver and renal function tests and serum mineral profile were analyzed at day 0, 7 and 21. Hematological parameters; Hemoglobin concentration (Hb), Hematocrit (PCV), Erythrocyte count, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Total Leukocyte Count (TLC), Differential Leukocytic Count (DLC), and platelet count were measured by automated hematological analyser (Abacus junior vet 5, Diatron, Austria). Serum biochemical parameters, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), blood urea nitrogen (BUN), and creatinine were analyzed using Pictus 700 chemistry analyzer (Diatron, Hungary).

Serum quantity of minerals (Cu, Zn, Na, K, Mg, Ca and P) was also determined at day 0 7, 21 and 35. Serum levels of zinc and copper were estimated by the method described by (Ali *et al*., 2013). Briefly, for wet digestion, a quantity of 0.5 mL serum and 5 mL nitric acid were taken in a digestion flask and was placed on hot plate at 90°C for 15 minutes until 2 ml material was left over. After cooling, 2.5 ml perchloric acid was added and heated until the volume was reduced to 2 ml, which was then stored in plastic bottles. These digested samples were used for the estimation of copper by atomic absorption spectrophotometer (Perkins Elmer Analyst 800, USA). Serum zinc level was estimated by using commercial kits in chemistry analyzer.

Determination of serum levels of sodium, potassium, magnesium, calcium, and phosphorus was done by automated blood chemistry analyzer hitachi 705 (Hitachi, Japan), using Thermo Fisher Scientific Inc. (USA) reagents.

Statistical analysis was done by using statistical package for social sciences (SPSS 16.0, SPSS, Chicago, USA). by one way ANOVA. The differences between diseased and healthy horses at different days were analyzed by Tukey's test (P value< 0.05).

RESULTS AND DISCUSSION

Haemolytic parameters

A comprehensive analysis of hematological parameters, liver and renal function indicators and mineral profile was performed at day 0, 7 and 21 of diseased and healthy horses to mark the changes in them. All the studied parameters were altered significantly at day 0, 7 and 21 (P<0.05). Table I shows changes in hematological parameters in diseased horses.RBC, Hb, PCV, MCHC and platelets showed significant decrease (P<0.05), while MCV, RDW, TLC, DLC increased significantly (P<0.05) during the course of disease.

Table I.- Hematological changes in diseased and healthy horses.

Parameters	Unit	Week 1		Week 3		Week 5	
		Diseased	Healthy	Diseased	Healthy	Diseased	Healthy
RBC	10^{12}	3.26±03*	5.89±0.04	3.28±0.03*	5.88±0.03	3.27±03*	5.84±0.03
Hb	g/dl	7.24±0.02*	11.87±0.03	7.21±0.02*	11.89±0.39	7.22±0.02*	11.93±0.04
PCV	%	24.62±0.03*	37.72±0.03	24.59±0.02*	37.75±0.04	24.60±0.03*	37.84±0.05
MCV	Fl	58.47±0.03*	41.42±0.04	58.41±0.03*	41.44±0.03	58.48±0.03*	41.53±0.04
MCH	Pg	23.74±0.03*	15.15±0.3	23.78±0.03*	15.17±0.03	23.76±0.03*	15.20±0.04
MCHC	g/l	25.12±0.03*	36.21±0.03	25.16±0.04*	36.41±0.03	25.20±0.03*	36.54±0.03
RDW	%cv	27.04±0.03*	19.35±0.04	27.11±0.03*	19.37±0.03	26.94±0.03*	19.45±0.04
TLC	10^{9}	14.42±0.04*	7.23±0.03	14.35±0.03*	7.25±0.04	14.52±0.04*	7.27±0.03
Neutrophils	%	57.32±0.04*	41.77±0.04	57.23±0.04*	41.72±0.03	57.39±0.04*	41.79±0.03
Basophils	%	1.12±0.03*	0.39±0.03	1.11±0.02*	0.38±0.03	1.11±0.03*	0.39±0.03
Eosinophils	%	21.57±0.04*	11.21±0.08	21.44±0.03*	11.19±0.07	21.61±0.04*	11.27±0.02
Lymphocyte	%	64.81±0.03*	45.61±0.03	64.88±0.04*	45.63±0.03	64.74±0.03*	45.75±0.03
Monocytes	%	4.37±0.03*	1.23±0.03	4.35±0.04*	1.25±0.02	4.31±0.03*	1.27±0.03
Platelets	10^{3}	145.21±3.85*	185.95±6.8	145.15±3.86*	184.84±7.06	145.33±3.8*5	185.78±6.51

*Significant difference between diseased and healthy horses (P < 0.05). Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; RBC, red blood cells; RDW, red cell distribution width; TLC, total leukocyte count.

These findings were in agreement with the studies of Pinna *et al*. (2013) and Oliviera *et al*. (2013), who reported a decrease in all mentioned parameters, because of hemolytic anemia caused by equine leptospiral infection. Same results were reported in dogs (Goldstein *et al*., 2006) and rats (Tonin *et al*., 2012). According to literature (Tonin *et al*., 2012), leptospirosis causes hemolytic anemia and capillary damage because of the hemolysin expressed by the bacteria. This caused a decrease in RBC, Hb, PCV, MCHC and increase in MCV and RDW. Cottle and Hughes (2010) reported same hematological alterations for hemolytic anemia, which is a frequent finding for equine leptospirosis.

Hematological analysis further revealed a significant increase in TLC and DLC, which is similar to the studies reported by (Oliviera *et al*., 2013) who reported same findings in equine leptospirosis, because leptospirosis is a bacterial disease and cause leucocytosis to combat bacterial infection (Goldstein *et al*., 2006; Lather *et al*., 2009; Pinna *et al*., 2010). The platelets were lesser in infected horses as compared to healthy ones which might be because of hemorrhages caused by the organism (Adler and Moctezuma, 2010) as pulmonary hemorrhage syndrome has been reported in *Leptospira* infected animals (Levett, 2011), humans (Amilasan *et al*., 2012) and dogs (Birnbaum *et al*., 1998).

Table II shows that liver enzymes; ALT, AST, ALP and TB increased significantly (P<0.05) at day 0, 7 and 21 in infected horses. Renal function parameters *i.e.* BUN and creatinine also showed significant increase (P<0.05) in diseased horses.

Same findings were reported by Pinna *et al*. (2010) and Adler and Moctezuma (2010) because of hepatocellular damage, caused by the leptospiral toxins during leptospiremia (Birnbaum *et al*., 1998). Levette (2001) reported elevated bilirubin and liver enzyme levels during leptospirosis which required many weeks for normalization which is in line with our findings.

BUN and creatinine were significantly increased because of renal damage caused by *Leptospira* (Sitprija, 2008). Adler and Moctezuma (2010) declared kidney as the colonization site during leptospirosis which may have caused renal damage, leading to increased BUN and creatinine in our study. Same abnormal changes in renal functions were reported by (Zala *et al*., 2014) during human leptospirosis.

Serum mineral profile was also analyzed during leptospiral infection and is presented in Table III. Sodium, potassium, calcium, magnesium, phosphorus, copper and zinc were decreased significantly (P<0.05) in horses with equine leptospirosis as compared to healthy horses.

Table II.- Serum biochemistry of diseased and healthy horses.

Parameters	Unit	Day 0		Day 7		Day 21	
		Diseased	Healthy	Diseased	Healthy	Diseased	Healthy
ALT	U/L	33.67±0.92*	25.37±0.52	33.62±0.75*	25.31±0.55	33.59±0.92*	25.39±0.50
AST	U/L	263.65±4.94*	227.95±1.99	263.81±4.8*	227.89±2.02	263.57±4.94*	227.85±1.94
ALP	U/L	552.31±4.39*	492.31±2.44	551.91±4.15*	492.24±2.35	552.34±4.39*	492.39±2.36
TB	mg/dl	1.32±0.04*	0.87±0.04	1.35±0.03*	0.85±0.03	1.39±0.04*	0.88±0.04
BUN	mg/dl	37.41±0.54*	29.19±0.90	37.35±0.48*	29.27±1.10	37.42±0.54*	29.41±0.67
Creatinine	mg/dl	1.98±0.04*	1.21±0.03	1.95±0.03*	1.19±0.03	1.94±0.04*	1.18±0.03

*Significant changes between diseased and healthy horses (P<0.05). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TB, total bilirubin; BUN, blood urea nitrogen.

Table III.- Serum mineral profile in diseased and healthy horses.

Parameters	Unit	Day 0		Day 7		Day 21	
		Diseased	Healthy	Diseased	Healthy	Diseased	Healthy
Sodium	mmol/l	99.20±0.61*	137.78±0.72	98.21±0.78*	136.35±0.81	99.28±0.61*	137.91±0.61
Potassium	mmol/l	2.12±0.04*	4.11±0.06	2.41±0.05*	4.07±0.06	2.13±0.04*	4.12±0.05
Calcium	mmol/l	2.51±0.02*	3.12±0.01	2.54±0.02*	3.15±0.02	2.56±0.02*	3.14±0.02
Magnesium	mmol/l	0.41±0.03*	0.780±0.05	0.39±0.03*	0.74±0.03	0.44±0.03*	0.77±0.03
Phosphorus	mmol/l	0.62±0.02*	1.12±0.04	0.67±0.03*	1.09±0.04	0.59±0.02*	1.15±0.04
Copper	mmol/l	11.25±0.55*	18.45±0.44	11.42±0.60*	18.43±0.42	11.29±0.55*	18.41±1.10
Zinc	mmol/l	7.21±0.25*	14.08±0.26	7.31±0.19*	14.15±0.25	7.17±0.25*	14.14±0.22

* Significant changes between diseased and healthy horses (P<0.05).

These clinico-pathological changes were notable during the course of disease and were consistent till the 21st day of analysis.

This is in agreement with the findings of Sitprija (2008) who reported low level of Ca, Mg, and P because of their abnormal excretion due to renal damage during leptospirosis. Low levels of Na and K were observed in our study, which favors the findings of Cerqueira *et al*. (2008) because of tubular defects in acute renal failure during leptospirosis, which may require recovery time of up to 6 months. Andrade *et al*. (2007) also reported *Leptospira* induced dysregulation of sodium transporters in kidneys. Tubular defects have been considered to be occurred because of inhibition of Na^{+}/K^{+} ATPase. Na^{+}/K^{+} pump is the driving force for reabsorption for potassium, which is inhibited by glycolipoproteins present in *Leptospira* (Ibrahim *et al*., 1997). This inhibitory effect causes potassium loss leading to lower potassium levels during leptospirosis. Sitprija (2008) described low levels of sodium as a sequel of increased levels of ADH during leptospiral invasion. Spichler *et al*. (2008) reported low levels of magnesium, same as found in our study, because of direct effect of leptospiral biochemical contents on proximal tubule and loop of Henle function. Same findings of low Na, K and Mg were reported in human leptospirosis by Araujo *et al*. (2010) because of proximal tubular injury, as this portion of nephron is main target for leptospiral colonisation. Proximal tubular injury decreases sodium and water reabsorption and increases sodium and water transport to the distal nephron, which may increase potassium excretion causing hypokalemia (Russell, 2000). Sitprija (2008) further reported decreased copper and zinc levels during tropical diseases as malaria and leptospirosis which is in agreement with the findings of our study. Low copper levels, indicating compromised immunity was reported in bovine leptospirosis by Mohammadi and Sakhaee (2015).

CONCLUSION

It is concluded that hemolytic anemia, liver and renal tubular damage impart significant clinico-pathological changes in hematology, serum biochemistry and mineral profile during equine leptospirosis.

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Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

Adler, B. and Moctezuma, A.D.P., 2010. *Leptospira* and leptospirosis. *Vet. Microbiol*., **140**: 287–296. https://doi.org/10.1016/j.vetmic.2009.03.012

Ali, F., Lodhi, L.A., Qureshi, Z.I., Ahmad, I. and Hussain, R., 2013. Serum mineral profile in various reproductive phases of mares. *Pak. Vet. J.*, **33**: 296-299.

Amilasan, A.T., Mugen, U., Motoi, S., Eumelia, S., Cecilia B.M.P., Nobuo, K., Kumiko, Y., Wolf-Peter, S., Shane, M., Efren, D.M., Benito, V.J. and Koya, A., 2012. Outbreak of leptospirosis after flood, the Philippines. *Emerg. Infect. Dis*., **18**: 91-94. https://doi.org/10.3201/eid1801.101892

Andrade, L., Rodrigues, A.C., Sanches, T.R.C., Souza, R.B. and Seguro, A.C., 2007. Leptospira leads to dysregulation of sodium transporters in the kidney and lung. *Am. J. Physiol. Renal Physiol*., **292**: 586-592. https://doi.org/10.1152/ajprenal.00102.2006

Araujo, E.R., Seguro, A.C., Spichler, A., Magaldi, A.J., Volpini, R.A. and Brito, T.D., 2010. Acute kidney injury in human leptospirosis: an immunohistochemical study with pathophysiological correlation. *Virch. Arch*., **456**: 367–375. https://doi.org/10.1007/s00428-010-0894-8

Balamurugan, V., Gangadhar, N.L., Mohandoss, N., Thirumalesh, S.R.A., Dhar, M., Shome, R., Krishnamoorthy, P., Prabudhas, K. and Rahman, H., 2013. Characterization of leptospira isolates from animals and humans: phylogenetic analysis identifies the prevalence of intermediate species in India. *Springerplus,* **2**: 362-370. https://doi.org/10.1186/2193-1801-2-362

Baverud, C., Gunnarasson, A., Engvall, E., Frazen, P. and Egenvall, A., 2009. *Leptospira* seroprevalence and associations between seropositivity, clinical disease and host factors in horses. *Acta Vet. Scand*., **51**: 15. https://doi.org/10.1186/1751-0147-51-15

Birnbaum, N., Barr, S.C., Center, S.A., Schermerhorn, T., Randolph, J.F. and Simpson, K.W., 1998. Naturally acquired leptospirosis in 36 dogs: Serologic and clinicopathological features. *J. Small Anim. Pract*., **39**: 231-236. https://doi.org/10.1111/j.1748-5827.1998.tb03640.x

Cerqueira, T.B., Athanazio, D.A., Spichler, A.S. and Seguro, A.C., 2008. Renal involvement

in leptospirosis – New insights into pathophysiology and treatment. *Braz. J. Infect. Dis*., **12**: 248-252. https://doi.org/10.1590/S1413-86702008000300016

Cottle, H.J. and Hughes, K.J., 2010. Haemolytic anaemia in a pony associated with a perivascular abscess caused by *Clostridium perfringense*. *Equine Vet. Educ*., **22**: 13-19. https://doi.org/10.2746/095777309X474

Goldstein, R.E., Lin, R.C., Langston, C.E., Scrivani, P.V., Erb, H.N. and Barr, S.C., 2006. Influence of infecting serogroup on clinical features of leptospirosis in dogs. *J. Vet. Intern. Med*., **20**: 489–494. https://doi.org/10.1111/j.1939-1676.2006.tb02886.x

Hamond, C., Pinna, A., Martins, G. and Lilenbaum, W., 2014. The role of leptospirosis in reproductive disorders in horses. *Trop. Anim. Hlth. Prod*., **46**: 1–10. https://doi.org/10.1007/s11250-013-0459-3

Himani, D., Suman, M.K. and Mane, B.G., 2013. Epidemiology of leptospirosis: an Indian perspective. *J. Fdborne Zoon. Dis*., **1**: 6-13.

Ibrahim, Y.M., Meyer, B.B. and Cheval, L., 1997. Na,K- ATPase: a molecular target for *Leptospira interrogans* endotoxin. *Braz. J. Med. Biol. Res*., **30**: 213-223. https://doi.org/10.1590/S0100-879X1997000200009

Kojouri, G.A., Taghadosi, C., Momtaz, H. and Taheri, E., 2009. A comparison between polymerase chain reaction and enzyme linked immunosorbant assay methods for detecting leptospira in equine recurrent uveitis. *J. Equ. Vet. Sci*., **29**: 795-798. https://doi.org/10.1016/j.jevs.2009.10.015

Lather, D., Saxena, V. and Mishra, S.K., 2009. Leptospirosis In a dog: a case report. *Haryana Vet*., **48**: 110-111.

Levett, P.N., 2001. Leptospirosis. *Clin. Microbiol. Rev.*, **14**. 296-326. https://doi.org/10.1128/CMR.14.2.296-326.2001

Mohammadi, E. and Sakhaee, E., 2015. Association between serum copper concentration and the risk of bovine leptospirosis. *Comp. clin. Pathol*., Online ISSN: 1618-565X.

Oliviera, E.R., Wercauteres, S.G., Gadelha, A. and Leite, A.K.R.D., 2013. Leptospirosis in equine: clinical and laboratory findings. *Rev. Cient. Eletrô. Med. Vet*., **11: 1-7**.

Pinna, A., Martins, G., Souza, G. and Lilenbaum, W., 2013. Influence of seroreactivity to *Leptospira* and reproductive failures in recipient mares of equine embryo transfer programs. *Reprod. Domest. Anim*., **48**: e55–e57. https://doi.org/10.1111/rda.12166

Pinna, M., Martins, G., Freire, I. and Lilenbaum, W., 2010. Seropositivity to *Leptospira interrogans* serovar Bratislava associated to reproductive problems without significant biochemical or hematological alterations in horses. *Ciênc Rural*., **40**: 2214-2217. https://doi.org/10.1590/S0103-84782010005000178

Russell, J.M., 2000. Sodium-potassium-chloride cotransport. *Physiol. Rev.,* **80**:211–276.

Saleem, M.H., Khan, M.S., Durrani, A.Z., Hassan, A., Ijaz, M. and Ali, M., 2013. Leptospirosis: An emerging zoonosis in Pakistan. *Pakistan J. Zool*., **45**: 909-912.

Sitprija, V., 2008. Altered fluid, electrolyte and mineral status in tropical disease, with an emphasis on malaia and leptospira. *Nat. Rev. Nephrol*., **4**: 91-101.

Spichler, A., Athanazio, D.A., Furtado, J., Seguro, A. and Vinetz, J.M., 2008. Severe, symptomatic hypomagnesemia in acute leptospirosis. *Am. J. trop. Med. Hyg*., **79**: 915-917.

Tilahun, Z., Reta, D. and Simenew, K., 2013. Global epidemiological overview of leptospirosis. *Int. J. microbiol. Res*., **4**: 09-11.

Tonin, A.A., Pimentel, V.C., Silva, A.S., Azevedo, M.I.A., Souza, V.C.G., Wolkmer, P., Rezer, J.F.P., Badke, M.R.T., Leal, D.B.R., Schetinger, M.R.C., Monterio, S.G. and Lopes, S.T.A., 2012a. Adenosine deaminase activity in serum, erythrocyte and lymphocytes of rats, infected with *Leptospira icterohaemorrhagiae*. *Res. Vet. Sci*., **92**: 197-201. https://doi.org/10.1016/j.rvsc.2011.01.013

Tonin, A.A., Silva, A.S.D., Azevedo, M.I.D., França, R.T., Paim, F.C., Schaefer, P.C., Martins, J.L.R., Badke, M.R.T. and Lopes, S.T.D.A., 2012. Hematologic and biochemical alterations in Wistar rats experimentally infected by *Leptospira interrogans*. *Comp. clin. Pathol*., **21**: 833–838. https://doi.org/10.1007/s00580-011-1186-7

Verma, A., Stevenson, B. and Adler, B., 2013. Leptospirosis in horses. *Vet. Microbiol*., **167**: 61-66. https://doi.org/10.1016/j.vetmic.2013.04.012

Zala, D.B., Khan, V. and Das, V.K., 2014. A study on few biochemical parameters of clinically suspected and laboratory confirmed Leptospirosis cases. *J. appl. Nat. Sci*., **6**: 12-13.