

## Investigation on Efficacy of a Commercial Vaccine for Treatment of Leptospirosis in Cattle

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**Abstract:** The aim of this study was to determinate leptospirosis in cattle and to investigate efficacy of a commercial vaccine in treatment of leptospirosis. Upon observing leptospirosis specific clinical signs including haematuria and mucosal icterus in 2 heifers and mouse fleashes on the surface of the water reserve of the farm, the clinicians tentatively diagnosed the diseased heifers as leptospirosis. In addition, 9 heifers had general clinical signs including fever, apathy and tachypnea. Blood samples were collected from 41 heifers kept in the farm, including those exhibiting specific and non-specific clinical signs. All clinically diseased heifers were seropositive for leptospirosis confirmed by Microagglutination Test (MAT). The antibody titers were 1/100 or above in 11 out of 41 heifers (26.8%). All 41 heifers were vaccinated with a specific commercial vaccine (Lepto Shield 5<sup>®</sup>) developed against leptospirosis. At the forth day of vaccination, one of the diseased heifers died of acute leptospirosis while the others clinically recovered 8 days after vaccination and none of the seropositive heifers showed any clinical signs of the disease. No side effects were observed within a month following vaccination. The results of the present study suggest that Lepto Shield 5<sup>®</sup> is effective not only for protection but also for treatment of leptospirosis in cattle.

**Key words:** Cattle, Lepto Shield 5<sup>®</sup>, leptospirosis, prognosis, treatment, vaccination

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### INTRODUCTION

Leptospirosis, a spirochetal zoonosis is a globally well-known infectious disease that can cause abortus, premature deliveries, infertility, failure in milk production and sudden deaths in cattle (Sambasiva *et al.*, 2003; Kocabiyik and Cetin, 2004). Leptospirosis has been reported worldwide leptospirosis seroprevalence among cattle is 24% (for serovar hardjo) and 10% (for serovar pomona) in Canada (Gregoire *et al.*, 1987) and 7.4% in Czech Republic (Tremel and Nesnalova, 1995), 45% in USA and Puerto Rico (Miller *et al.*, 1991).

The seroprevalence in Turkey is 8.04% however, the reported seroprevalence rate in Kars and Ardahan Provinces was much higher 33.6% (Sahin *et al.*, 2000). *L. hardjo* and *L. grippotyphosa* pathogenic serotypes are most common and well known in Turkey (Kocabiyik and Cetin, 2004).

Alive or dead rodents are reservoirs of leptospirosis (Kocabiyik and Cetin, 2004). Transmission of infection occurs through either direct or indirect contact with urine or tissues of infected animals (Sakhaee *et al.*, 2007). Upon

contact with infected materials, *Leptospira* sp. spread the body through cuts and abrasions in the skin and penetrate into mucous membranes (Sambasiva *et al.*, 2003; Kocabiyik and Cetin, 2004; Sakhaee *et al.*, 2007; Salinas-Melendez *et al.*, 2007; Aslantas and Ozdemir, 2005). Acute leptospirosis especially pomona serotype is quite dangerous for calves. High fever, anorexia, apathy, dyspnea, hemorrhage, icterus, hemoglobinuria and hemolytic anemia are commonly observed in infected calves (Sambasiva *et al.*, 2003; Sakhaee *et al.*, 2007). Body temperature suddenly increases up to 41°C. Acute syndrome is not typical in leptospirosis induced by Hardjo serotype but morbidity and mortality are higher in calves (Salinas-Melendez *et al.*, 2007). In progressed ages, diagnosis of the disease becomes difficult as the clinical signs of the disease change dramatically.

In lactating cows, milk yield decreases and milk content changes and contains blood cells and a number of somatic cells. Dairy cow's udder is soft and hanging (Ertas *et al.*, 2005). Abortus can be observed sporadically in chronic form of the disease (Tremel and Nesnalova, 1995; Ertas *et al.*, 2005). *Leptospira* sp. are invasive to

genital organs following 6-12 weeks of transmission. The abortus incidence is observed usually during the last trimester of gestation. The infected pregnant cows can deliver premature calves with a low survival rate (Ertas *et al.*, 2005). Acute cases are treated successfully with tetracycline (Anom. 1; Anom. 2) Oxytetracycline, erythromycin, enrofloxacin, tiamulin, tilozin are the other treatment choices for acute cases.

In chronic cases, oxytetracycline, amoxicillin, enrofloxacin are preferred (Aslantas and Ozdemir, 2005). The antibiotic treatment in cows has well recognized disadvantages such that antibiotic residues in milk bear some risks for human health (Ertas *et al.*, 2005). Therefore, much safer treatment options should always be investigated. In this study, researchers aimed to investigate the efficacy of a vaccine in protection and treatment of leptospirosis in cattle.

#### MATERIALS AND METHODS

**Blood serum samples:** Blood samples were collected from 41 heifers, two of which exhibited specific clinical signs of leptospirosis including hematuria and mucosal icterus. Nine additional heifers also exhibited common clinical signs including fever, apathy and tachypnea. Blood samples were collected into sterilized and vacuumed tubes and transported in cold chain the Laboratory of Leptospirosis in the Etlik Institute for Research and Diagnosis, Ankara. The serum samples were obtained and stored at -20°C until assayed with Micro Agglutination Test (MAT).

#### Serological tests

**Standard strains and control sera:** In order to prepare MAT antigens, 4 strains of *L. interrogans* were used: serovar hardjo Prajitno, serovar grippotyphosa Moscow V, serovar icterohaemorrhagiae RGA and serovar Pomona. Reference strains including *L. hardjo*, *L. grippotyphosa* and *L. icterohaemorrhagiae* as well as the antisera of *L. pomona* and negative control sera were obtained from The Central Veterinary Control and Research Institute.

**Micro Agglutination Test (MAT):** MAT test was applied according to the instruction provide by Cole *et al.* (1973). Double fold dilutions of serum samples were prepared with sterile PBS starting with 1/100. Antigen's of living leptospira serovares added to the each dilution equally than taken to the incubation at 30°C for 3 h.

Antibody titer of each serum was defined as the last dilution which has 50% agglutination and lyses. The sera with 1/100 or above titers were accepted as seropositive.

**Immunization procedure:** All 41 heifers including those with clinical signs were vaccinated with a specific a commercial vaccine (Lepto Shield 5<sup>®</sup>) developed against leptospirosis. Each heifer received 2 mL of vaccine administered intramuscularly.

#### RESULTS AND DISCUSSION

**Clinical findings:** Eleven heifers in a farm exhibited clinical signs including apathy, fever and tachypnea. Among the 11 heifers, two exhibited leptospirosis specific clinical signs, mucosal icterus and hematuria. In addition, the water reservoir in the farm had mouse fleshes on the surface.

**Serological findings:** Using MAT, antibody titres against leptospirosis were determined in 11 out of 41 (26.8%) heifers. The data on the antibody titres are shown in Table 1.

#### Vaccination protocol and subsequent clinical observation:

All 41 heifers in the farm were vaccinated with a commercial vaccine (Lepto shield5<sup>®</sup>, Egevet). About 2 mL of vaccine were administered intramuscularly. On the 4th day of vaccination, an infected heifer with specific signs died of acute leptospirosis. The remaining clinically diseased and seropositive heifers including the other heifer with acute leptospirosis recovered. The specific and non-specific clinical signs disappeared by the 8th day of vaccination.

Leptospirosis is an infectious disease of the cattle causing fever, anorexia, dyspnea, icterus, hemoglobinuria, hematuria, hemolytic anemia, renal failure and sudden death (Sambasiva *et al.*, 2003; Kocabiyik and Cetin, 2004; Sahin *et al.*, 2002; Bomfim *et al.*, 2008). In the study, two clinically diseased heifers exhibited leptospirosis specific signs, hematuria and mucosal icterus while the other 9 seropositive heifers expressed the general clinical

Table 1: Serum antibody titers in seropositive heifers of Leptospirosis

No. of infected cattle	Serotypes			
	H	G	I	P
1	1/100	1/200	-	-
2	1/100	-	-	-
3	1/100	-	-	-
4	1/100	-	-	-
5	1/100	1/400	-	-
6	1/100	1/400	-	-
7	1/100	1/200	-	-
8	1/100	1/100	-	-
9	1/100	1/200	-	-
10	1/100	-	-	-
11	1/100	-	-	-

H: *Leptospira interrogans* serovars Hardjo; G: *Leptospira interrogans* serovars Grippotyphosa; I: *Leptospira interrogans* serovars Icterohaemorrhagiae; P: *Leptospira interrogans* serovars Pomona

signs such fever, apathy and tachypnea. We think that the infection in these 9 heifers had just started and the intervention prevented the progression of the disease to more specific signs. The majority of the Leptospirosis cases are diagnosed by serology and the reference standard assay is the microscopic agglutination test MAT. ELISA, IFAT, Dark Field Microscopy (DFM) and PCR methods are available for diagnosis (Bulu *et al.*, 1990; Ozdemir, 1994). In the study, researchers employed MAT to confirm leptospirosis. Among the test 4 serovars of leptospires sp., we determined the serovars *L. hardjo*, *L. grippityphosa*. These serovars commonly found in Turkey. In the treatment of leptospirosis, specific antibiotics including tetracycline, oksitetracycline, erythromycin, enrofloxacin, tiamulin and tilozin are often used (Aslantas and Ozdemir, 2005; Ertas *et al.*, 2005) (Anom. 1; Anom. 2). In the study, researchers preferred a commercial vaccine for the choice of treatment. Disappearance of clinical signs of the disease by the 8th day of vaccination suggests that the vaccine is also effective in the treatment of leptospirosis in cattle and a good alternative to antibiotics. However, the mechanism of vaccine treatment should be further investigated. Researchers think that the inactive Lepto-Shield 5<sup>®</sup> polivalent vaccine further increases T cell response in seropositive animals.

### CONCLUSION

In this study, the preliminary results obtained through the present study suggest that the Lepto-Shield 5<sup>®</sup> polivalent vaccine is effective not only in the treatment but also prevention of leptospirosis in cattle. However, the mechanism of vaccine treatment should be further investigated.

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### REFERENCES

Aslantas, O. and V. Ozdemir, 2005. Determination of the seroprevalence of leptospirosis in cattle by MAT and ELISA in Hatay, Turkey. *Turk. J. Vet. Anim. Sci.*, 29: 1019-1024.

Bomfim, M.R.Q., E.F.B. Stancioli and M.C. Koury, 2008. Detection of pathogenic leptospires in urine from naturally infected cattle by nested PCR. *Vet. J.*, 178: 251-256.

Bulu, A., R. Dorteller, O. Ozkan and F. Hosturk, 1990. Dogu Anadolu'nun bazı illerinde sigir ve koyunlarda leptospirosis vakalari, yayilisi ve serotipleri üzerine araştırma. *Etlik Vet. Mikrobiyol. Derg.*, 6: 49-60.

Cole, J.R., R.S. Catherine and A.R. Pursell, 1973. Improved microtechnique for the leptospiral microscopic agglutination test. *Appl. Microbiol.*, 25: 976-980.

Ertas, H.B., B. Cetinkaya, A. Muz, H. Ongör, V. Ozdemir and N. Yazicioglu, 2005. Sigirlarda *Leptospira seroprevalansinin* mikroskopik aglutinasyon testi (MAT) ve ELISA ile saptanması. *Turk. J. Vet. Anim. Sci.*, 26: 1415-1420.

Gregoire, N., R. Higgins and Y. Robinson, 1987. Isolation of Leptospires from nephritic kidneys of Beef cattle at slaughter. *Am. J. Vet. Res.*, 48: 370-371.

Kocabiyik, A.L. and C. Cetin, 2004. Bovine leptospirosis in South Marmara region of Turkey: A serological survey. *Rev. Med. Vet.*, 155: 606-608.

Miller, D.A., M.A. Wilson and G.W. Beran, 1991. Survey to estimate prevalence of *Leptospira interrogans* infection in mature cattle in the United States. *Am. J. Vet. Res.*, 52: 1761-1765.

Ozdemir, V., 1994. Türkiye' de Leptospirozisinin Dagilimi ve Serotiplendirilmesi Üzerine Bir Çalışma. I. Ulusal Vet. Mikrobiyol. Kongresi, Ankara.

Sahin, M., F. Aydin, R.V. Ozdemir, O. Genç and M.A. Guler, 2000. Kars ve Ardahan Illerinde Sigir Leptospirozisinin Kültürel ve Serolojik Yöntemlerle Arastirilmesi. IV. Ulusal 13. Vet. Mikrobiyol. Kongresi, Ankara, 48.

Sahin, M., F. Aydin, V. Ozdemir, O. Genç and M.A. Guler, 2002. Kars ve Ardahan Illeri'nde sigir Leptospirozisinin serolojik yöntemlerle arastirilmesi. *Turk. J. Vet. Anim. Sci.*, 26: 17-25.

Sakhaee, E., G.H.R. Abdollahpour, M. Bolourchi, A.M. Tabatabayi and S. Tabrizi, 2007. Serologic and bacteriologic diagnosis of bovine leptospirosis in Tehran suburb dairy farms. *Iran. J. Vet. Res.*, 8: 325-332.

Salinas-Melendez, J.A., C. Narvaez-Arce, V. Riojas-Valdes, A. Cantu-Covarrubias, R. Avalos-Ramirez and J.C. Segura-Correa, 2007. Seroprevalance of leptospirosis in beef cattle of nuevo leon, Mexico. *J. Anim. Vet. Adv.*, 6: 1265-1268.

Sambasiva, R.R. G. Naveen, P. Bhalla and S.K. Agarwal, 2003. Leptospirosis in India and the rest of the World. *The Brazilian J. Infect. Dis.*, 7: 178-193.

Trembl, A. and E. Nesnalova, 1995. Leptospirosis in slaughter cattle-serological and bacteriological examinations. *Vet. Med. Czech*, 40: 305-309.