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Immunohistochemical detection of leptospiral antigens in cases of naturally occurring abortions in sheep

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Abstract

This study was carried out to determine the leptospiral antigens in naturally occurring 108 abort sheep fetuses. To determine the antigen localizations in tissue sections (kidney, liver, lung and spleen) of each fetus were stained with immunoperoxidase (IP) technique and then were examined under light microscope. The results of this study showed that 19 (17%) out of 108 fetuses were positive for the presence of leptospiral antigens. In the 19 positive cases, leptospiral antigens were found in lung ($n = 10$; 9%), liver ($n = 7$; 6%), kidney ($n = 12$; 11%) and spleen ($n = 2$; 2%) samples. Microscopic studies demonstrated that leptospiral antigens were located in the cytoplasm of macrophages in interalveolar and interlobular septum of the lung; in the cytoplasm of macrophages in the portal regions and hepatocytes of the liver; in the cytoplasm of epithelial cells of renal pelvis, in the cytoplasm of epithelial cells of cortical and medullar tubules, and macrophages of intertubular region in the kidney. In the spleen, antigens were detected in the cytoplasm of macrophages throughout the parenchymal tissue. In conclusion, the results of this study demonstrate that leptospirosis could be a major disease causing abortions in sheep.

Keywords: Leptospirosis; Aborted sheep fetuses; Immunohistochemistry

1. Introduction

Leptospirosis is an important infectious disease of livestock animals and humans caused by serovars of Leptospira interrogans. It is particularly important due to causing abortion and stillbirth in farm animals. *Leptospira pomana*, *L. hardjo* and *L. grippotyphosa* have been the most common serovars isolated from sheep (Ellis et al., 1983; Bulu et al., 1990; Maxie, 1993). Because the leptospires die readily in tissues or body fluids, diagnosis of leptospirosis in individual animals is often difficult (Ellis et al., 1983; Maxie, 1993). Leptospirosis may be diagnosed by the presence of leptospiral antigens in tissue sections. A variety of silver staining, immunofluorescence, immunoperoxidase (IP) and immunogold silver techniques are common laboratory techniques for diagnosis (Ellis et al., 1983; Scanziani et al., 1989, 1991). An IP staining procedure to detect leptospiral antigens in tissues has been shown to be an accurate and reproducible technique and be able to be used on formalin-fixed and paraffin-embedded tissues. Also, there is a positive correlation between results

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obtained from culture studies and results obtained from the IP staining methods on infected tissues (Ellis et al., 1983).

The IP method is demonstrably more sensitive than silver staining tissue samples (Scanziani et al., 1989, 1991; Szeredi and Haake, 2006) and more specific than serology performed using the microscopic agglutination test. Compared with culture, the sensitivity of the IP was 78% and its specificity was 100% (Scanziani et al., 1989).

Objective of this study was to determine the presence of leptospiral antigens in tissue sections in naturally occurring abort sheep fetuses by using IP technique.

2. Materials and methods

Totally, 108 ovine aborted fetuses were necropsied. All the samples were collected from Erzurum and Van provinces (of eastern Turkey). Abortions had been come from the different flocks and regions, and the number of abortions in the flocks had not been known. Tissue samples from liver, lung, spleen and kidney of each fetus were taken for histological and IP examinations. These samples were routinely processed staining for haematoxylin and eosin, and Levaditi-Manovelian as well as IP (Ellis et al., 1983; Scanziani et al., 1991).

IP staining was performed with the avidin–biotin peroxidase complex procedure (Ellis et al., 1983; Falini and Taylor, 1983), using commercial IP kits (Shandon, Standard Sensitivity Cadenza Tags, Peroxidase Kit with AEC-catalog no: 407300). Hyperimmune serum for *L. grippotyphosa* and *L. hardjo* serotypes were supplied from Etlik-Ankara Central Veterinary Control and Research Institute (CVCRI)-Leptospirosis lab. IP staining procedure was performed with manual immunostaining equipment (Sequenza Immunostaining Center-Shandon).

For the IP staining procedure, optimum temperature and duration were detected as 37 °C and 1 h, respectively. Also, suitable dilutions of hyperimmune serums were used for *L. grippotyphosa* as 1/2000 and *L. hardjo* 1/1600. The best suitable staining of case and control preparations were attained at the temperature and dilutions mentioned previously. Against to each serotype of two leptospira species (*L. grippotyphosa* and *L. hardjo*) were determined cross-reactions with hyperimmune sera and were obtained similar results. After that, all tissues were stained with control preparations using IP staining procedure to detect leptospiral antigens in tissue sections.

3. Results

In IP staining, the presence of leptospiral antigens were detected in 19 (17%) out of 108 ovine abort fetuses. Leptospiral antigens were found in lung ($n = 10$; 9%), liver ($n = 7$; 6%), kidney ($n = 12$; 11%) and spleen ($n = 2$; 2%) tissue samples (Table 1). These IP positive abortive fetuses had come from the different flock.

Table 1
Pattern of IP positive organs in the fetuses with leptospiral abortion

Pattern of leptospiral positive IP reaction product	Number
Lung only	3
Liver only	3
Kidney only	5
Spleen only	1
Liver, lung and kidney	3
Lung and kidney	3
Liver, spleen, lung and kidney	1
Total	19

Microscopic studies demonstrated that the leptospiral antigens were located in the cytoplasm of macrophages in interalveolar and interlobular septum of the lung; in the cytoplasm of macrophages in the portal regions and hepatocytes of the liver; in the cytoplasm of epithelial cells of renal pelvis; in the cytoplasm of epithelial cells of cortical and medullar tubules, and macrophages of intertubular regions in the kidney (Fig. 1A and B). The same antigens were determined in the cytoplasm of macrophages throughout the parenchymal tissue in spleen. In these tissues, staining generally was focally observed and tended to be granular. The immunoreaction was particularly strong in the tubular and renal pelvis epithelial cells.

4. Discussion

Diagnosis of leptospirosis in animals usually depends on serological examination in the live animal or a combination of histopathology and culture in the dead animal (Ellis et al., 1983). The frequencies of leptospirosis were reported in sheep as 3% (Bulu et al., 1990) and 4% (Ozkan et al., 1993) with serological tests in Erzurum region. Recently, some local studies in eastern Turkey show that the seroprevalences of leptospirosis in cattle were found to be 36% (Sahin et al., 2002), 38% (Genc et al., 2005) and 14% (Aslantas and Ozdemir, 2005). In a national survey, the seroprevalences of leptospirosis were found to be 8% in cattle and 8% in sheep (Ozdemir and Erol, 2002). Both in local studies and in national survey, hardjo and grippotyphosa were the most prevalent serovars. In the same province, Turkutanit et al. (2002) reported that the presence of leptospiropsis in sheep was 9% at tissue sections stained with Levaditi-Manovelian method, whereas Saglam et al. (2003) reported that the presence of the leptospirosis in cattle was 41% at tissue sections processed by

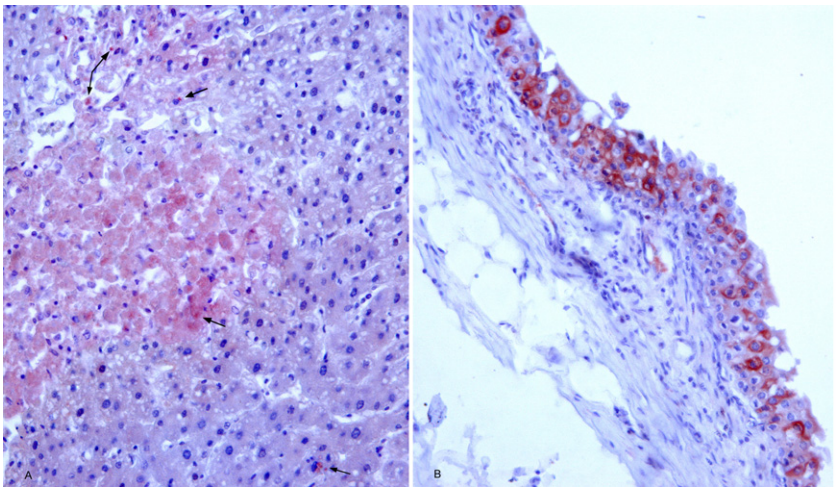

Fig. 1. (A) Leptospiral antigens in cytoplasm of hepatocytes (arrows) (320×) (avidin–biotin peroxidase method, Mayer's haematoxylin counterstain). (B) Leptospiral antigens showing the strong immunopositivity in the cytoplasm of epithelial cells of renal pelvis in the kidney (300×) (avidin–biotin peroxidase method, Mayer's haematoxylin counterstain).

IP staining technique. Also Temur and Saglam (2003) detected that the incidence of leptospiral antigens in aborted bovine fetuses was 8 (24%) out of 33. In the present study, 108 ovine abort fetuses were examined by using IP technique and 19 (17%) of them were leptospirosis positive. Nevertheless, it has been reported that leptospiral antigens are generally located in liver, kidney and lungs in animals (Maxie, 1993; Saglam et al., 2003). In this study, leptospiral antigens were detected in spleen as well.

L. hardjo was found at slaughter in the kidneys of three seropositive ewes, but not in uterus or salpinges of these animals (Cerri et al., 1996). In IP staining, leptospiral antigens were especially determined to be in the renal tubular lumen and the luminal surface of tubular cells, and intensely brown in color (Ellis et al., 1983; Scanziani et al., 1989, 1991). Previous studies (Ellis et al., 1983; Falini and Taylor, 1983) showed that optimum temperature was 37 °C, not a room temperature, and optimum incubation period was 20–30 min for immunohistochemical staining. Also temperatures below 37 °C may reduce the reaction activity (Ellis et al., 1983; Falini and Taylor, 1983). Results presented in this study are well in agreement with the literatures. Ellis et al. (1983) reported that the staining reaction could be abolished by increasing dilution of the hyperimmune serum. In this study, there were dilution effects on the intensity of staining. Reproducible results were observed at a dilution of 1/2000 for *L. grippotyphosa* and 1/1600 for *L. hardjo*. Cross-reactivity of a polyclonal antiserum to a given serovar with other serovars occurs with immunostaining procedures (Scanziani et al., 1991). However, broad-spectrum reactivity of the IP procedure might be useful in the generic diagnosis of leptospires (Scanziani et al., 1991). Identification of the serogroup or serovar is obtainable by the use of different specific antisera at the final dilution or by monoclonal antibodies (Ellis et al., 1983). Hence we thought that antibodies against *L. harjo* and *L. grippotyphosa* might allow the detection of other serovars as well.

In conclusion, the IP technique can be useful and rapid for the diagnosis of leptospirosis. Liver, kidney, lung and spleen were suitable organs for diagnosis. Despite it is reported that leptospirosis in sheep is not a major problem in Turkey (Ozdemir and Erol, 2002), but according to the results of the present study, leptospirosis is one of very important causes of ovine abortions in eastern region of Turkey. Proper health surveillance as part of animal husbandry management is very crucial. When leptospirosis is diagnosed during the early phase, further abortion losses may be reduced. On the other hand in these areas vaccination and eradication studies are recommended against leptospirosis for prophylactic purposes.

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