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Research Article

DETECTION OF LEPTOSPIROSIS AND LISTERIOSIS IN BOVINE ABORTION: AN IMMUNOHISTOCHEMICAL STUDY

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The present study was conducted to decipher Leptospira and Listeria associated abortion in bovine using immunohistochemical techniques. Immuno-histochemical staining for antigens of *Leptospira interrogans* in fetal kidney revealed positive staining of organisms preferentially in lining tubular lumen and within tubular epithelial cells. Listerial antigen was identified as principally intracellular (in neutrophils and macrophages) evident as deep brown, short rods and/or clusters of organism(s) within the abscesses and area of necrotic foci. Immuno-histochemical method can be used as a rapid tool in the diagnosis of Leptospira and Listeria abortion cases.

Key words: Abortion, Immunohistochemistry, Leptospirosis, Listeriosis

Leptospirosis is a worldwide zoonotic disease caused by pathogenic *Leptospira* species. Infectious abortion caused by *Leptospira* species is a significant cause of reproductive failure resulting in enormous economic losses in bovine in tropical and subtropical countries (Elis, 1984). It particularly causes abortion and stillbirth in farm animals. Abortion may occur several weeks after infection of the dam will be the only evidence of the disease in this form (Adler and Moctezuma, 2010). *Leptospira* serovar *hardjo* is a potential cause of disease and should be considered when investigating cases of abortion, stillbirth, or atypical mastitis in dairy cows. ELISA has been described previously for detection of *Leptospira* serovar h*ardjo* (Adler *et al.*, 1982; Berchovich *et al.*, 1990; Richardson *et al.*, 1995; Theirmann and Garrett, 1983) in cows. Leptospirosis may be diagnosed by the presence of leptospiral antigens in tissue section (Ellis *et al.*, 1983). Listeriosis is caused by a ubiquitous, zoonotic, intracellular pathogen *Listeria monocytogens* characterized by encephalitis, septicaemia and abortion in animals. Transmission of the disease occurs

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through ingestion of feed contaminated with infected fetus, placenta or uterine discharge (Yaeger and Holler, 2007). Vertical transmission is the usual source of infection in ruminants, infections being transmitted transplacentally. *L. monocytogenes* gains access to the fetus by hematogenous penetration of the placental barrier. Abortion usually occurs in late pregnancy (after 7 months). Serological tests for the detection of antibodies have not been traditionally used for the diagnosis of listeriosis. A number of diagnostic tools have been tried and they have all been found to be largely unreliable, lacking sensitivity and specificity. Isolation of *L. monocytogenes* may require weeks and have low sensitivity (OIE, 2014). In this study, Immuno-histochemical technique was used for confirmation of *L. monocytogenes* and Dot ELISA was used for *Leptospira* serovar *hardjo* antibody detection in serum samples of aborted animals and immuno-histochemistry for the presence of leptospiral antigens in aborted fetal tissues from natural field (clinical) cases of bovine abortion.

MATERIALS AND METHODS

Tissue samples from the aborted fetuses of cattle and buffaloes (n=21) and placental cotyledons (n=14) were collected from cases of abortion at farms from various districts of Punjab in 10% neutral buffered formalin for routine histopathology. The tissue samples (lung, liver, kidney, brain) were dehydrated, cleared and embedded in paraffin sections (4-5 µm thick) were cut and stained with haematoxylin and eosin (H&E) as per standard protocol (Luna, 1968). Blood sampling was possible only from 10 aborted animals from which aborted fetuses were also collected. The sera were separated and stored at -20°C until they were tested for antibodies to *Leptospira hardjo* using ImmunoComb® Bovine Leptospira Antibody Test Kit (Biogal–Galed Labs, Israel)

For immuno-histochemical studies 4-5 µm thick paraffin embedded tissue sections were cut and mounted on Superfrost Plus, positively charged microscopic slides (Fisher Scientific, USA). Immuno-histochemical staining was performed by using advanced SS™ One-Step Polymer–HRP IHC Detection System (BioGenex Laboratories Inc., San Ramon, California, USA). A commercially available polyclonal antibody against *Leptospira interrogans* (ABD serotac) and *Listeria monocytogenes* (ABD serotac) were used in a dilution of 1:500. As negative control, sections were incubated with PBS instead of the primary antibody.

RESULTS

In the present study, out of ten serum samples collected from the aborted animals, two were positive for *Leptospira* serovar *hardjo* antibodies as analyzed by Dot ELISA. Moreover, gross and histologic changes in placenta were non-specific. Positive immunoreactivity was seen in kidney of two fetuses. Immuno-histochemical staining for antigens of *Leptospira interrogans* in a formalin-fixed, paraffin-embedded kidney section of aborted fetus revealed positive staining of organisms lining tubular lumens and of antigens within tubular epithelial cells (Fig. 1).

Detection of Leptospirosis and Listeriosis in bovine abortion

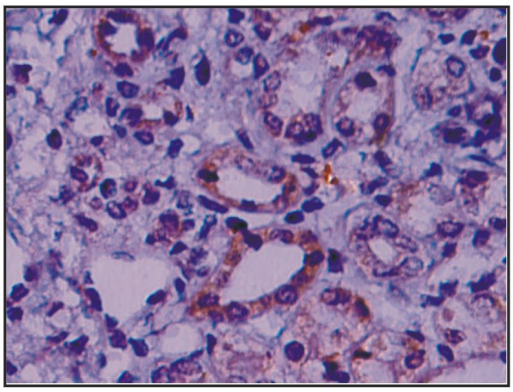

Fig. 1. Kidney: Immuno-reactivity to the anti- *Leptospira* polyclonal antibody (IHC-DAB- Gill's Haematoxylin counter stain x100)

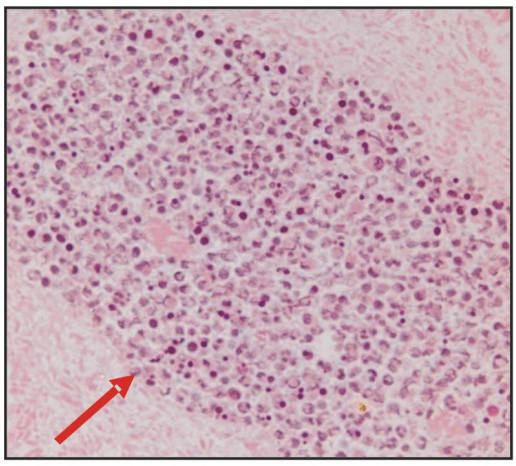

Fig. 2. Liver:Arrow showing abscess in aborted foetal liver (H &E, 40X)

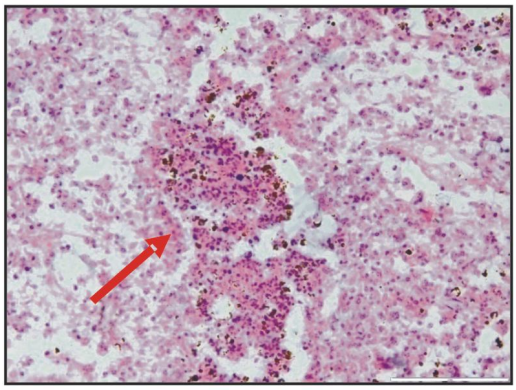

Fig. 3. Lung: Arrow showing abscess in aborted foetal lung (H &E, 20X)

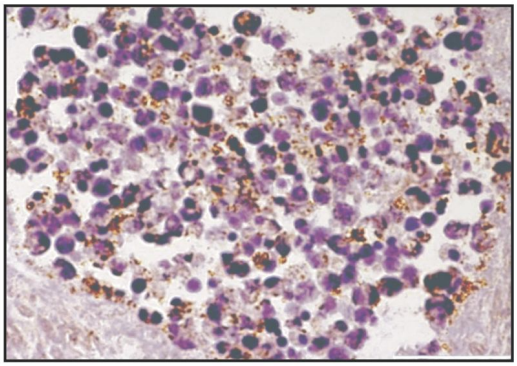

Fig. 4. Lung : Immuno-reactivity to the anti- *Listeria monocytogenes* polyclonal antibody with typical morphology of short rods in the abscess (IHC-DAB- Gill's Haematoxylin counter stain x100)

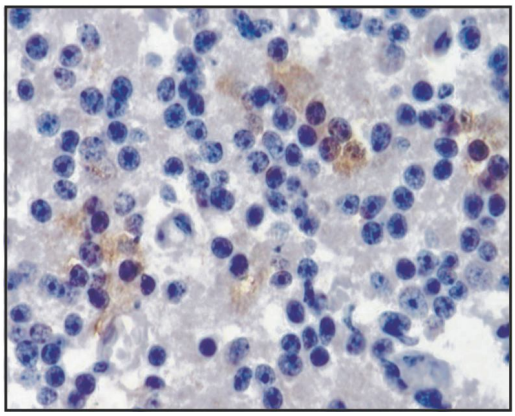

Fig. 5. Brain: Immunoreactivity to the anti-*Listeria monocytogenes* poly*clonal* antibody (100X)

Listerial abortions were observed in four aborted fetuses at eighth month of gestation. Multifocal areas of abscesses and acute to sub-acute inflammation in liver and lung (Figs. 2 & 3) of aborted foetuses were observed. These foci have a central area of necrosis in which the organism can be visualized along with small numbers of degenerating neutrophils and mononuclear cells. Listerial antigen positive debris was identified as principally intracellular (in neutrophils and macrophages) deep brown short rods and clusters of organisms within abscess (Fig. 4) and necrotic foci in lung. In two of the aborted foetuse(s), glial nodules were seen in section of brain which showed immuno-reactivity to anti- *Listeria monocytogenes* polyclonal antibody (Fig. 5).

DISCUSSION

Diagnosis of leptospirosis is usually based on the demonstration of serum antibodies with serological test like the microscopic agglutination test (MAT) and enzyme-linked immunosorbent assay (Levett, 2001). In the present study, Dot ELISA revealed two serum samples positive for *Leptospira* serovar *hardjo* antibodies collected from the ten aborted animals. Histopathological changes were observed mainly in the foetal kidneys where the organism arrives via the bloodstream. In the kidneys, the *leptospira* species multiplies, causing tubule-interstitial lesions (Scanziani *et al*., 1989). Immunoperoxidase staining of *Leptospira* spp. has been shown to be an accurate and reproducible technique to detect leptospiral antigen on formalin-fixed and paraffin-embedded tissues (Ellis *et al*., 1983). Moreover, there is a positive correlation between results obtained from culture studies and results obtained from the IHC staining methods on infected tissues (Ellis *et al*., 1983). In the present study, leptospiral antigens intensely brown in color were detected in lining renal tubular lumen and within tubular epithelial cells by immuno-histochemical techniques as reported earlier by various authors. (Ellis *et al*., 1983; Scanziani *et al*., 1989) Further, Saglam *et al.* (2008) found 11% of leptospiral antigen in kidney of aborted sheep foetuses by immunoperoxidase (IP) technique.

Confirmation of listerial infection in the laboratory is currently based on combined diagnostic assays like histological examination, immuno-histochemistry and bacteriological isolation (Gasanov *et al*., 2005; Low and Donachie, 1997). Isolation of *L. monocytogenes* may be unsuccessful even when appropriate samples are submitted as culture-negative cases can be associated with few or no bacteria in the lesions. Various authors demonstrated immunohistochemistry (IHC) as a helpful tool for diagnosis in natural cases of encephalitis (Johnson *et al*., 1995; Campero *et al*., 2002). In the present study, positive immunoreactivity to listerial antigen was seen in section of fetal lung, liver and brain (Fig. 5). Moreover, Weinstocks *et al.* (1995) described immunohistochemical testing useful in locating antigen in lesions with few bacteria or bacterial antigens in culture-negative cases for confirming the diagnosis of encephalitic listeriosis.

It is concluded that immuno-histochemical method can be used as a rapid tool in the suspected leptospira and listera abortion cases, when isolation is not possible or material is fixed in formalin.

Conflict of interest: Authors declare that there is no conflict of interest regarding the present research work.

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