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Histopathological and Immunohistochemical Studies on Field Samples of Uterus, Placenta and Some Visceral Organs Collected from Ewes and Goat Naturally Infected with *Mycoplasma capricolum Capripneumoniae*

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Abstract: The purpose of this study was determining the existence and distribution of *Mycoplasma Capricolum Capripneumoniae* (*Mccp*) bacteria in uterus and placenta of pregnant ewes and goats by using immunohistochemistry (IHC) protocol; more over describing the histopathological changes in uterus, placenta and some visceral organs. Field samples from 30 ewes and 12 goats (4 ewes and 3 goats were pregnant) collected from Giza governorate, Egypt and examined for *Mccp* infection. Indirect Latex Agglutination Test (LAT) was used for detection of *Mycoplasma* anti-Capsular Polysaccharide antibody (CPS). 25% and 20% was showed positive reaction in goats and ewes respectively. High intensity of (CPS) antigen was detected in chorioallantoic trophoblast cells of placenta; also detected in infiltrated macrophages of endometrium of non-pregnant ewes and goats. Low intensity of antigen was observed in lung and liver. Most of LAT positive cases were showed severe ulcerative and suppurative endometritis, suppurative interstitial and alveolar pneumonia. Suppurative enteritis also observed. Severe vacuolar degeneration and necrosis were observed in liver and heart. The obtained results gave a conclusion as; *Mccp* able to pass through placental barrier and has a great affinity to live in chorioallantoic trophoblast cells; also found in macrophages aggregated in endometrium with low affinity toward visceral organs.

Key words: Ewes · Goats · IHC · LAT · *Mccp* · Pathology

INTRODUCTION

Mycoplasma capricolum capripneumoniae (*Mccp*) is one of the *Mycoplasma mycoides* clusters [1]. *Mccp* considered as the causative agent of contagious caprine pleuropneumonia disease (CCPP) which is fatal caprine disease [2]. OIE considered CCPP as one of the notifiable diseases causing maximum threat to goat farming industry in developing countries [3]. The rate of morbidity and mortality in Mccp infection is 100% and 60–80% respectively [4]. It is highly endemic in Africa, Middle East and Asia [5, 6].

Latex Agglutination Test (LAT) was designed to detect serum antibodies against the mycoplasma capsular polysaccharide antigen (CPS) for *Mccp* diagnosis [7]. LAT is field, rapid and inexpensive test for CBPP diagnosis. It was recently identified as a top priority by the OIE and FAO Consultative group on CBPP [8].

The main pathological lesion of CCPP is fibrinous pleuropneumonia with increase straw yellow pleural fluid in infected lung [4]. Lung of *Mccp* infected animal was suffered from suppurative pneumonia which transformed into a voluminous abscess and become hepatized in texture as a consequence of secondary bacterial infection [9, 2].

The purpose of study was to determine the existence and distribution of *Mccp* CPS antigen in uterus and placenta of pregnant ewes and goats; also in uterus of non-pregnant cases by immunohistochemistry (IHC); more over describing the histopathological changes in uterus, placenta and some visceral organs.

MATERIAL AND METHODS

Examined Animals and Collected Samples: Field cases of total number 30 ewes and 12 goats (4 ewes and 3 goats

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were pregnant in late stage) aged from 1- 3 years were used. They were collected from different herds in Giza governorate, Egypt. These cases previously examined for *brucella* infection before postmortem examination by Rose Bengal test which were exhibited negative results. Serum was examined for presence of anti-*mycoplasma capricolum* antibody by LAT. Tissue samples of uterus, placenta, lung, liver, heart and intestine were examined for presence of *Mccp* CPS antigen by IHC and for histopathological changes.

Latex Agglutination Test (LAT): Blood samples were collected from jugular vein just before slaughtering for separation of serum. LAT kit was imported from veterinary laboratory Agency CO. United Kingdom for detection of anti-CPS antibody of *Mccp*. Add 15µl of tested sera to 15µl of *Mccp* (Capri LAT) antigen coated latex beads in room temperature. Mix the reagents in the plate. Positive agglutination ranged from heavy to light flocculation precipitate [10]. Positive and negative control samples were also enclosed in the test.

Histopathology: Uterus, placenta, lung, heart, intestine and liver were taken from the LAT positive cases. It was fixed in 10% neutral buffer formalin (NBF) for 24 hr. Tissues were processed, embedded in paraffin wax, sectioned at 5 ìm and stained with hematoxyline and eosin [11].

Immunohistochemistry (IHC): Immunohistochemical staining with streptavidin biotin peroxidase complex technique was carried out in paraffin embedded tissue samples of the cases gives positive results by LAT. Epitope retrieval was occured by pretreatment of tissue with Proteinase K (0.1%) at 37ºC/15 minute. Detection of the CPS antigen in examined cases was done by incubation of tissues with monoclonal anti CPS mouse antibody as a primary antibody imported from Thermo Lab Co. India. Peroxidase detection kit imported from Scy Tek Lab. USA was used for detection of the primary antibody. Species specificity of kit is anti-mouse, anti-rabbit. DAB chromogen as a color indicator was applied [12].

RESULTS

Latex Agglutination Test (LAT): A total number 6 over 30 ewes (20%) and 3 over 12 goats (25%) were showed positive reaction in LAT. Only two pregnant cases one in ewes and other in goats exhibited strong positive results as showed in Table (1).

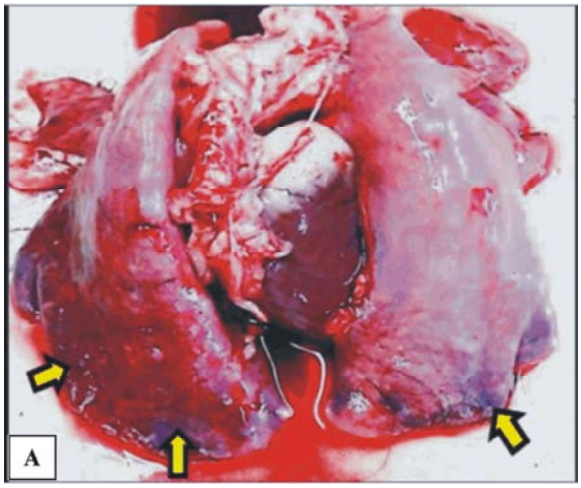

Fig (A): Severe pneumonia in caudal lobs of lungs with hepatization in texture (arrows).

Table 1: Latex Agglutination Test variations in positive cases:

Case No.	Speicies	AGE/ years	Intensity of reaction	Physiological status
138	Ewe	3	+++	Pregnant
136	Ewe	3	++	Non pregnant
157	Ewe	4	+	Non pregnant
70	Ewe	4	+	Non pregnant
81	Ewe	3	+	Non pregnant
6	Ewe	1	+	Non pregnant
132	Goat	1	++	Non pregnant
103	Goat	2	+++	Pregnant
68	Goat	2	+	Non pregnant

Heavy precipitate **+3,** less heavy precipitate +2, Light precipitate +1.

Pathological Findings

Macroscopical Findings: The most pronounced macroscopical lesions in *Mccp* infected goat is severe pneumonia in the caudal lobs of lung which was hepatized in texture (Fig. A).

Histopathological and Immunohistochemical Findings

Uterus: Suppurative and ulcerative endometritis in 3 ewes and 2 goats from non-pregnant positive cases were seen which showed severe multifocal ulceration in endometrium. Moderate neutrophilic infiltration in lamina propria submucosa was seen (Plate 1-1). Pregnant ewe and goat although showed normal progestational proliferation but also exhibited severe exfoliation of uterine gland epithelium into lumen accompanied with massive neutrophilic accumulation. Severe congestion of uterine blood vessels was also seen (Plate 1-2). IHC findings in non-pregnant uterus; Moderate intracytoplasmic immunoreaction against CPS *Mccp* antigen in aggregated macrophages in the lamina propria

Histopathological and Immunohistochemical Findings:

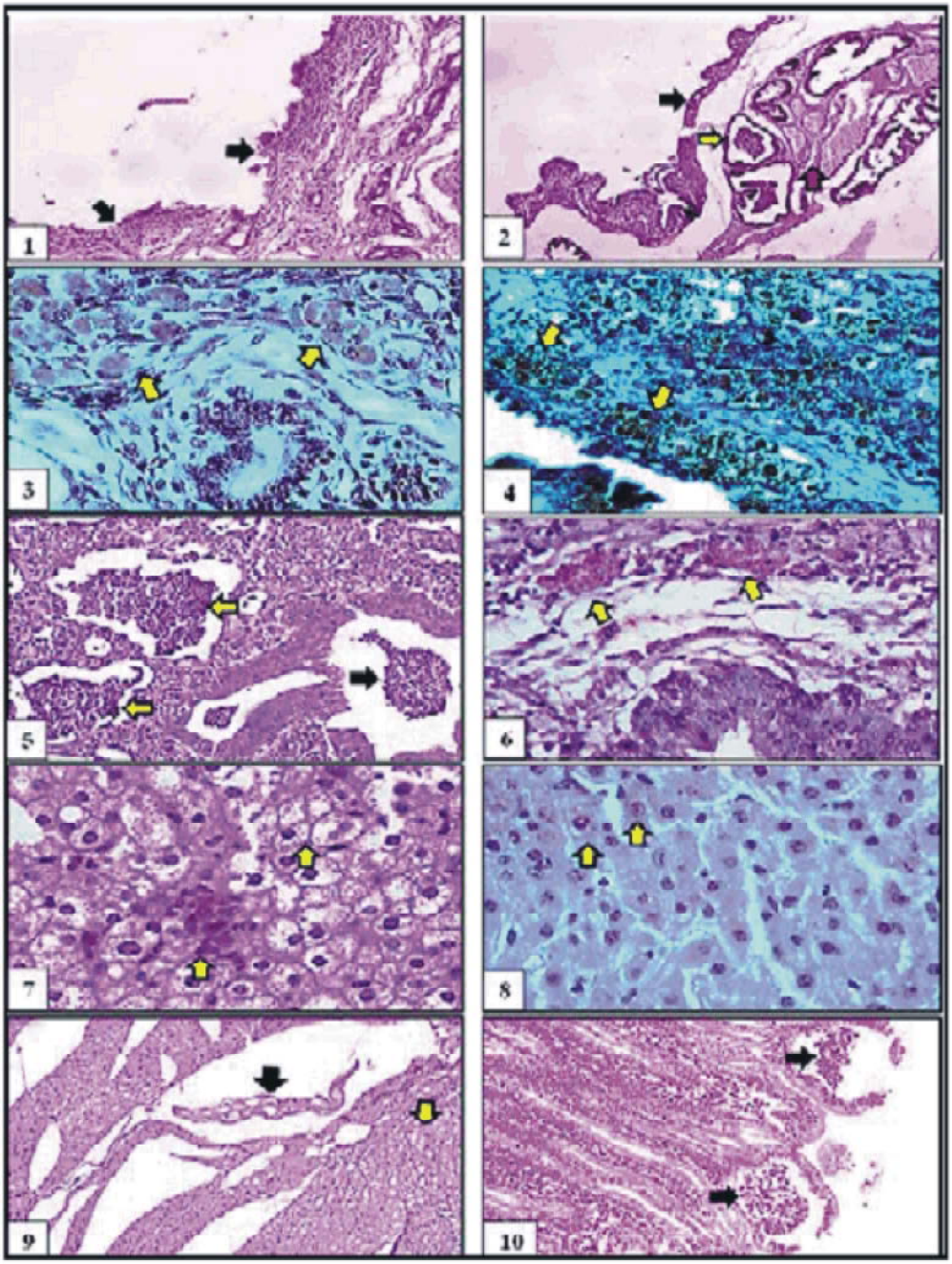

Plate 1: Uterus; non-pregnant ewe, showing multifocal exfoliated endometrial epithelium and moderate neutrophilic infiltration in lamina propria (arrows), H and E X 100. (2): Uterus; Pregnant goat, revealed severe intraluminal neutrophilic aggregation in uterine glands (yellow arrows), endometrial blood vessels severely congested (red arrows), H and E X 100. (3): Uterus; non-pregnant goat, showing brown immunostained *Mccp* CPS antigen in endometrialy infiltrated macrophages (arrows), IHC DAB chromogen X 400. (4): Placenta; goat, exhibited severe diffused golden brown *Mccp* CPS antigen in chorioallantoic trophoblast cells (arrows), IHC DAB chromogen X 400. (5): Lung; goat, showing severe diffused interstitial, intra-alveolar (yellow arrows) and intra-bronchial infiltration of neutrophils and macrophages (Black arrow), H and E X 200. (6): Lung; goat, revealed peribronchial faint brown positive immunoreaction against *Mccp* CPS antigen was found in lung tissue (arrows), IHC DAB chromogen X 200. (7): Liver; ewe, showing hyperactivity of Kupffer cells (black arrow) displayed in between degenerated hepatocytes was found (yellow arrow), H and E X 400. (8): Liver; ewe, showing faint immunostained *Mccp* CPS antigen in few hepatocytes was displayed (arrow), IHC DAB chromogen X 400. (9): Heart; goat, exhibited severe vacuolar degeneration in cardiac myocytes (black arrow) and purkinje fibers was detected (yellow arrow) H and E X 100. (10): Intestine; ewe, showing ulceration in the lining epithelium of intestinal villi associated with diffusely aggregated neutrophils in the core of villi was observed (arrow), H and E X 200.

of endometrium was seen (Plate 1-3). Placenta in one eweand one goat revealed that; CPS antigen was diffusely detected in most trophoblast cells of chorioalantoic villi (Plate 1-4). More over this antigen was found intra-cytoplasmic in aggregated macrophages in uterine caruncle of ewe and goat.

Lung: 4 ewes and 2 goats from the all nine LAT positive cases were showed severe chronic interstitial and intra-alveolar suppurative pneumonia. Massive aggregation of macrophages and neutrophils in the interstitial areas leading to their thickening was observed. The purulent exudate occludes most of the bronchial lumen (Plate 1-5). Moderate intensity of distinct immunostaining granular material of *Mccp* antigen was found intercellular in a free form in the stroma of peribronchial spaces (Plate 1-6).

Liver: all nine positive cases showed diffuse vacuolar degeneration and necrosis in most of hepatocytes. Multifocal hyperactivity in Kupffer cells was aggregated in periportal area (Plate 1-7). Hepatocytes were showed mild positive intracytoplasmic immunoreaction against CPS antigen (Plate 1- 8).

Heart: Severe diffuse vacuolar degeneration in cardiac myocytes in four ewes and two goats. In addition; the purkinje fibers were showed degeneration and necrosis (Plate 1-9).

Intestine: Suppurative enteritis in 4 ewes and 2 goats were exhibited desquamation in epithelium lining of the apical portion of intestinal villi accompanied with neutrophilic infiltration in the core of villi (Plate 1-10).

DISCUSSION

Mccp is belongs to a group of five closely related ruminant pathogenic *Mycoplasma mycoides* cluster [1]. It was difficultly identified by routine methods [5, 13] where *Mccp* was shared in similar serological cross reactions and similar biochemical features that leading to the erroneous diagnosis of CCPP [14].

Mccp has been recorded to infect only goat and does not infect sheep [9]. In contrast to these findings; *Mccp* has been isolated from healthy sheep that came in contact with CCPP-positive goats in Africa [15]. Our finding agrees with this record; the percentage of positive goats was 25% and 20% in sheep by LAT. *Mccp* bacteria can replicate intracellularly and infect both of domestic and wild breeds of goat causing 100% morbidity and 60–80% mortality rates [4, 16].

LAT considered as field, reliable and more sensitive test than Complement Fixation Test (CFT) where; it detects serum *Mccp* antibodies in CCPP-infected goats using whole blood or undiluted serum [17]. In early infection the CPS antigen can eclipse the antibody response therefore false-negative result for CBPP with the CFT was occurred [7].

The histopathological findings showed severe ulcerative and suppurative endometritis in pregnant and non-pregnant positive cases, suppurative pleuropneumonia, vacuolar degeneration and necrosis in liver and heart. These findings could be clearly explained as; *mycoplasmas* infection was leading to inhibition of host cell catalase enzyme; subsequently intracellular accumulation of toxic metabolic products as hydrogen peroxide and superoxide occurred leading to phospholipid oxidation and cellular damage of host cells. Macrophage was the main inflammatory cells aggregated in infected tissues in our findings that could be clarified as; *Mycoplasmas* activates macrophages and stimulate cytokine production [18]. More over other studies carried out on placenta infected with *Mccp* which revealed diffuse, suppurative, necrotic placentitis [19]. Suppurative pneumonia in our study comes in agreement with López [20] who reported that; lung of infected goats showed muco-purulent exudates and active alveolar inflammation with macrophages prevalent over neutrophils. *Mycoplasma* infection leading to increase the chemical mediators as tumor necrosis factor alpha (TNFá), interleukin 1 (IL-1), IL-6, IL-8 and prostaglandin E2 which has a direct cytotoxic effect on endothelial cells and causing tissue damage [21].

IHC in my study pronounced the intra-cytoplasmic existence of *Mccp* CPS antigen in macrophages aggregated in endometrium of non-pregnant cases. More over it was intensively detected in choriallantoic trophoblasts of placenta in infected pregnant cases. These findings could be evidenced as; IHC confirm the existence of *Mccp* CPS antigens in choriallantoic trophoblasts and in the lumen of blood vessels in the allantoic membrane [19]. Low intensity of *Mccp* CPS antigen in lung was compatible with other studies reported that; the agent from the lung pneumonia due to *M. capripneumoniae* infection was not grown from

attempts to cultivate *M. capripneumoniae* from pulmonary or pleural lesions late in experimental infection [22].

CONCLUSION

The obtained results gave a conclusion as; *Mccp* can be gain access to placenta. IHC confirm this fact by detection of *Mccp* CPS antigen in chorioallantoic trophoblast cells; also found in macrophages aggregated in endometrium. Low affinity toward visceral organs was observed. We need further investigations as In Situ PCR to confirm the distribution and the cellular affinity of *Mccp* toward different tissues and systems.

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