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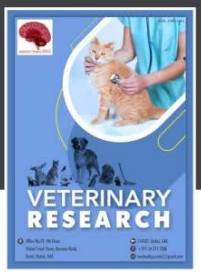

Study the Secondary Pathological Lesions

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Abstract: Snuffles disease is a major infectious disease process in rabbits caused by *Pasteurella multocida*. The importance of the Lipopolysaccharides (LPS) and Outer Membrane Protein (OMP) in the immunization and pathogenesis of the disease. This study aimed to identify all the pathological findings in rabbits immunized with LPS and OMP. For this reason a local strain of *Pasteurella multocida* were used and their LPS and OMP were extracted and purified and used for immunization of rabbits. Following immunization and inoculation of a challenge dose of the *Pasteurella multocida*, the bacterial Isolation were mild to moderate at 4th, 8th day and mild at 16th day post inoculation compared to heavy bacterial isolates in the control non immunized group. Also, more localized and granulomatous lesion were seen in immunized groups compared to extensive and diffuse septicemic lesions in non immunized control group.

INTRODUCTION

Pasteurellosis remain a disease of economic importance affecting wide range of animals specieces caused by the *Psteurella multocida* which is a primary or secondary agent in the disease process (Merza, 2008). Members of this species of bacterial agent colonize the upper respiratory tract of the most of animal specieses (Hirsh *et al*., 1990). Lipopolysaccharides and outer membrane proteins are the major part of the cell wall of *Psteurella multocida* and other gram negative bacteria, play a critical role in the pathogenesis of disease (Wijewardana, 1992). This bacterial agent is associated with a major disease problem in rabbits (snuffles disease); atrophic rhinitis, pneumonia, septicemia and death (Jaglic *et al*., 2004). Through the importance of this disease, this study aimed at.

Study the *Psteurella multocida* dissemination in the organs of rabbits following their immunization with lipopolysaccharides and outer membrane protein of *Pasteurella multocida* and under the effect of challenge dose of these bacteria.

Study the secondary pathological findings following rabbit immunization and under effect of challenge dose of *Psteurella multocida.*

MATERIALS AND METHODS

Psteurella multocida strain was supplied by AlKindi company for veterinary drugs and vaccine production, Baghdad, Iraq. This bacterial agent was reidentified to be sure *Psteurella multocida* using cultural, biochemical, API-20 Kit (Biomerieux-USA) (Wilson *et al*., 1993) extracted and purified (Chandan *et al*., 1994; Morrison and Leive, 1975) and outer membrane protein were also extracted and identified (Choi-Kim *et al*., 1991). LD50 for LPS and OMP were identified (Dixon, 1980). The 3 groups of rabbits (15 rabbits for each group) were taken. The first

group were immunized with LPS (30g kg rabbit^{-1}) the 2nd group were immunized with OMP 25g kg rabbit^{-1} and the 3rd group were injected with phosphate buffer saline. Immunization occurred at two doses, 2 weeks intervals and at 30th day post Immunization, all the rabbits groups were injected with a challenge dose of *Psteurella multocida* multocida (1×10^2 CFU mL^{-1}). At 4, 8 and 16th, bacterial isolation were done from the different organs (Wilson *et al*., 1993) and pieces of tissue from the different affected organs were taken and kept in neutral buffered formalin (10%), processed routinely for histopathology (Luna, 1968).

RESULTS AND DISCUSSION

The dissemination of *Psteurella multocida* in different organs of rabbits following immunization with LPS and OMP: *Psteurella multocida* were mild to moderatly isolated from the organs of immunized rabbits (LPS, OMP) at 4th, 8th day post inoculation compared to non immunized group which showed heavy isolation of *Psteurella multocida* from the different organs (Table 1).

The most bacterial Isolates were from the liver and lungs. At 16th days post inoculation, there is not isolation of *Psteurella multocida*from the different organs of immunized rabbits with LPS and OMP except that there is very mild bacterial isolation from lungs, liver and kidneys compare to heavy bacterial isolation from all the organs of non immunized control group of rabbits.

Our data revealed that the best protective status after the challenge dose in rabbits immunized with LPS and OMP of *Pasteurella multocida* resulted from higher humoral and cell mediated immune response induced in rabbits following their immunization with LPS and OMP which lead to decrease the number of bacterial colonies isolated from the internal organs of immunized rabbits following using challenge dose of *Pasteurella multocida*, so, the decline in the number of bacterial isolates related to high levels of humoral and cell mediated immune response induced by the LPS and OMP, in addition to the localized granulomatous lesions and localized inflammatory response induced following bacterial challenge which paly a critical role in clearing the internal organs from *Pasteurella multocida* (Raetz and Whitfield, 2002) following immunization with LPS of rabbits A similar findings reported by Bhattacharjee *et al*. (2002), whereas the non immunized group showed extensive bacterial isolation from the extensive distructive lesions and from the dead animals in this non immunized group.

The pathological findings: The lesions were more localized in the internal organs of immunized groups of rabbits (LPS and OMP) compared to non-immunized control group of rabbits which showed diffuse septicemic lesions in the different organs of rabbits. So, the immunized groups (LPS and OMP) showed:

The lungs: Showed extensive hyperplasia of the peribronchial associated lymphoid tissue (BALT) (Fig. 1), thickening of the alveolar septae due to their infiltration with mononuclear cells (Lymphocytes, macrophages and plasma cells) and congestion of alveolar capillaries, especially, at 4, 8th day post challenge and at 16th day focal aggregation of these mononuclear cells leading to early granulomatous lesions.

Table 1: Bacterial Isolation from the internal organs of rabbits immunized with LPS and OMP and following challenge with P. multocida

Groups	Days	Animal No.	Spleen	Liver	Kidney	Lung	Heart	Brain
LPS group	4th	1	++	++	++	++	+	-
		2	+	++	+	++	-	-
		3	+	++	+	++	-	-
	8th	1	+	++	+	++	-	-
		2	+	++	+	++	-	-
		3	+	++	+	++	-	-
	16th	1	-	+	+	+	-	-
		2	-	-	-	-	-	-
		3	-	+	-	+	-	-
OMP group	4th	1	++	++	++	++	+	+
		2	++	++	+	++	-	-
		3	+	+	+	+	-	-
	8th	1	++	++	++	++	+	+
		2	++	++	+	++	-	-
		3	+	+	+	+	-	-
	16th	1	-	+	-	+	-	-
		2	-	+	+	+	-	-
		3	-	-	-	-	-	-
Control infected group	4th	1	++++	++++	++++	++++	++	++
	8th	+++	+++	+++	+++	+++	++	+
	16th	+++	+++	+++	+++	+++	++	+

- (negative) + mild (1-5) colonies) ++ moderate (6-10 colonies) +++ heavy (10-20 colonies and above)

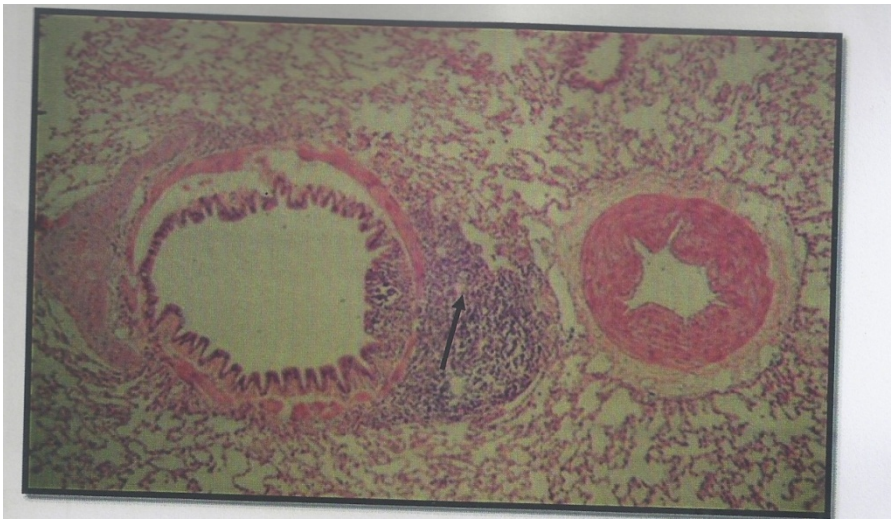

Fig. 1: Histological section of rabbit lung from LPS group at 16 days post challenge; showed BALT hyperplasia (→) (H&E X10)

Fig. 2: Histological section of rabbit liver from OMPs group at 16 days post challenge showed localized perivascular mononuclear cells cuffing around the central veins mainly macrophages and lymphocytes (→) (H&E X10)

The liver: Showed mononuclear cells aggregations in the liver parenchyma and prominence of kupffer cells at 4th, 8th days post challenge and at 16th days these mononuclear cells aggregation together with fibrosis in liver parenchyma lead to early granulomatous lesions (Fig. 2).

The kidneys: Showed hypercellularity of the glomeruli and dilation of the Bowman's space (Fig. 3) and mononuclear cells and neutrophils aggregations between the glomeruli and the renal tubules at the 4th, 8th day post challenge and at 16th days post challenge these mononuclear cells aggregations together with fibroblast proliferation lead to early granulomatous lesion formation.

The brain: Showed perivascular mononuclear cells cuffing and congestion and perineuronal edema along the post challenge period (Fig. 4).

The spleen: Showed extensive lymphoid hyperplasia at the white pulp regions (T cell and B cell regions) (Fig. 5) and hyperplasia of reticuloendothelial cells lining the red pulp along period of post challenge.

The lymph node: Showed hyperplasia of cortical lymphoid tissue with new germinal center formation (secondary lymphoid follicles formation) due to mononuclear cells (lymphocytes, macrophages and plasma cells aggregation) (Fig. 6) other organs, heart, trachea, testis, ovary, peritoneum showed only

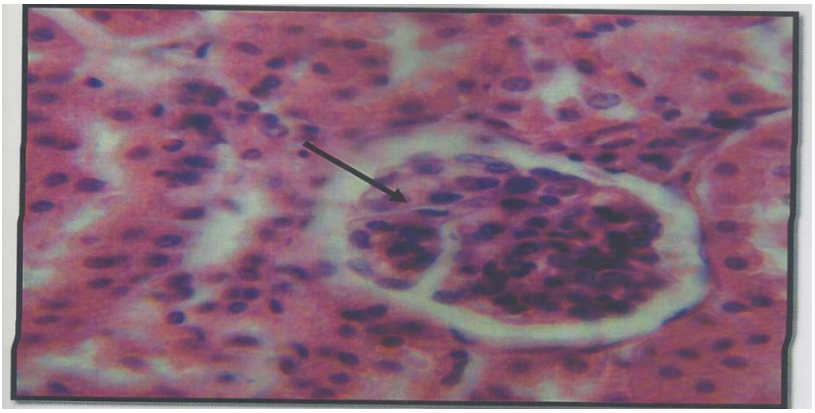

Fig. 3: Histological section of rabbit kidney from LPS group at 8 days post challenge; notice dilatation Bowman's space (→) (H&E X40)

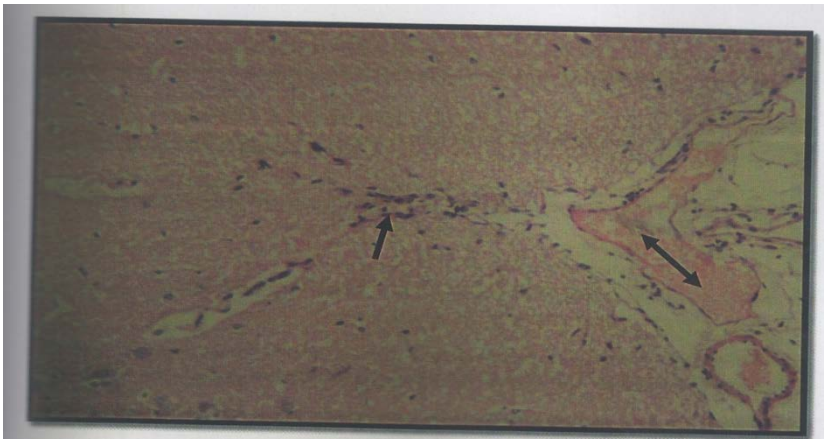

Fig. 4: Histological section of rabbit brain from immunized LPS group 4 days post challenge; showed congestion of blood vessels meninges (↔) and edema (→)(H&E X10)

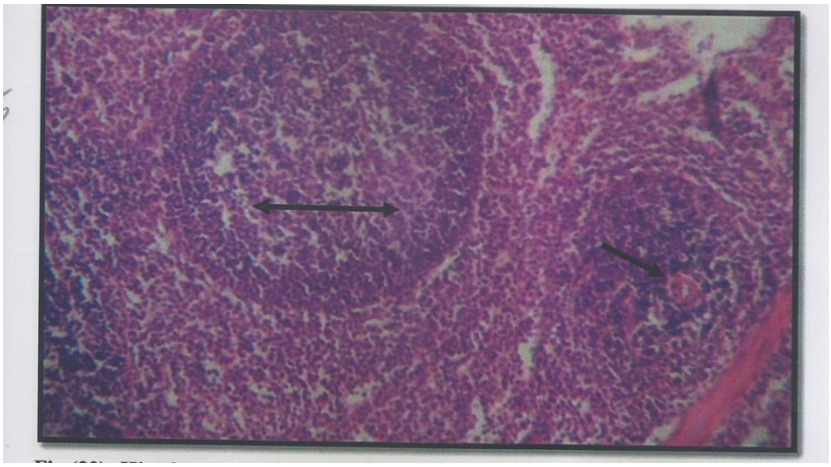

Fig. 5: Histological section of rabbit spleen from LPS group 8 days post challenge; revealed extensive hyperplasia of white pulp in arterial sheath region (T- cell region) (↔) and hyperplasia of remainder region of white pulp (B-cell region) (↔) (H&E X10)

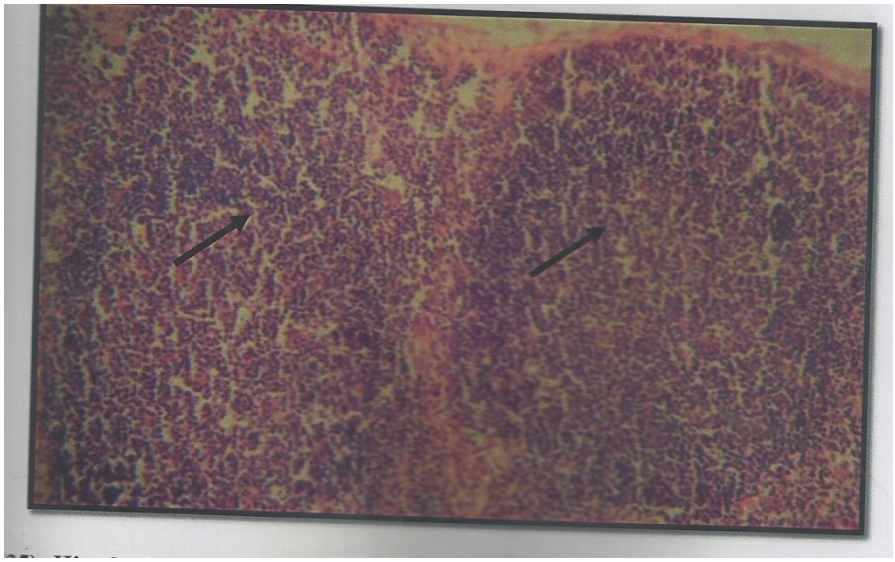

Fig. 6: Histological section of rabbit lymph node from LPS group at 16 days post challenge showed reactive hyperplasia of lymphoid follicles in cortical region (↔) (H&E X10)

congestion. All the organs of non immunized control group of rabbits showed extensive and diffuse septicemic, purulent lesions and death of the all animals in this group post challenge.

The most lesions in the immunized animals (LPS and OMP groups) were mononuclear cells aggregation together with early granulomatous lesions in the different organs of immunized groups and challenged with *Pasteurella multocida*, the localization of the lesions resulted from higher immune response (humoral and cellular immune response) induced by LPS and OMP immunization which lead to localization and granulomas formation following the challenge dose (Ko *et al*., 2002; Dannenberg *et al*., 1972) and decrease in the bacterial isolation in the different organs in addition to bacterial clearance (Raetz and Whitfield, 2002; Bhattacharjee *et al*., 2002; Tizzard, 1992). Hyperplasia of the lymphoid tissue in the lymph node, these lymphoid hyperplasia occurred as a result of immunization of the rabbits with LPS and OMP which induce higher immune response in these animals and resulted in to lymphoid hyperplasia (Tizzard, 1992). Other important lesion such as prominence and hyperplasia of kupffer cells in liver of immunized rabbits with LPS and OMP will indicate that the activated kupffer cells reduce the number of bacterial colonies in the liver and resulted in to localized and granulomatous reaction (Abbas *et al*., 1994) under the effect of humoral and cell mediated immune response induced by LPS and outer membrane protein.

CONCLUSION

Other lesions, congestion and the thrombosis of some blood vessels occurred as a result of adherence of platelets, red and white blood cells to the endothelial cells of blood vessels with the expression of adhesion molecules under the effect of bacterial toxins which reduced in immunized groups (Pawlinski *et al*., 2003).

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