

Role of IL-1 and IL-8 in placentitis in aborted cows infected with Salmonellosis in Babylon province

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Abstract

Hundred and twenty aborted placentas were obtained from aborted cows cultured directly and other were fixed in formaline buffer to study the effect of IL-1 and IL-8 in pathogenesis of Salmonella associated with placentitis in aborted cows. Results showed that 12 placentas were obtained from all abortion cases and there is high expression of IL-1 and IL-8 in 12 aborted placenta infected with Salmonella as a compared with noninfected placentas.

Keywords: Salmonella, aborted cows , placentitis , IL-1 and IL-8.

الخلاصة:

تم جمع 120 عينة مشيمة من ابقار مجهزة , زرعت العينات مباشرة وثبتت باستعمال محلول الفورمالين لمعرفة تأثير كل من الانترلوكين (8) و(1) على امراضية جرثومة السالمونيلا المصاحبة لالتهاب المشيمة في الابقار المجهزة . اظهرت النتائج تعبير عالي لكل من الانترلوكين (8) و(1) في 12مشيمة من مشائم الابقار المجهزة و المصابة بالسالمونيلا مقارنة بغير المصابة .

الكلمات المفتاحية : السالمونيلا , الابقار المجهزة , التهاب المشيمة , انترلوكين 1 و 8

Introduction

Salmonella, a Gram-negative, non-sporing, catalase-positive, oxidase-negative facultative anaerobic bacilli is a significant cause of morbidity and mortality in humans and animals,(Al-Wardi, A.H.; Al-Faidhi, E.A. and Said, J.K. (2004),(Barrow, P.A. (2007), with multidrug resistant *S. Enterica* serovar Typhimurium being an emerging problem. Contaminated food of animal origin, particularly meat products from cattle and pigs, is an important source of *S. Typhimurium* human infections (Dahlfors, R. A.; Buchan ,A.M.J. and Finlay, B.B. (1997). *S. Typhimurium* has been described as a collection of variants that vary significantly in their host range and their degree of host adaptation (Galan, J.E. (1996). It is the third most common serovar causing human food-poisoning in some parts of the world(Hassani ,L.; and AiatMelloul. A. (1999). As pathogens, they have developed complex virulence mechanisms to evade host defence mechanisms,Salmonella are primarily involved in reproductive disease in cattle(Oliveira, C.J.B.; Carvalho, L.F.O.S.; Fernandes, S.A.; Tavechio, A.T.; Domingues, F.J.Jr. (2005). *Salmonell adublinis* usually associated with enteric infection and diarrhea, particularly in calves, although infection of pregnant cows may result in abortion as the only clinical manifestation of infection in the herd. It is generally accepted that Salmonella infections elicit both cell-mediated and humoral immunereactions (Pie, S.; Truffa-Bachi, P.; Pla, M.; and Nauiel, C. (1997). The goal of this study was to evaluate the profile of cytokines responses in pathogenesis of placenta infected with Salmonella.

Materials and methods:

1.Aborted cows:

Hundred and twenty aborted cows were used in this study

2.Placenta specimens:

Placentas specimens were obtained from aborted cows divided into two parts, First part was cultured directly on MacConky agar (Difico), Salmonella selective medium S.S.agar, Xylose lysine dextrose agar and brilliant green, Oxoid). The cultures

were incubated at 37 C° for 2 days, aerobically . Second part of placenta tissue was fixed in 10% buffered formalin, processed routinely, and stained with haematoxylin and eosin (H&E).

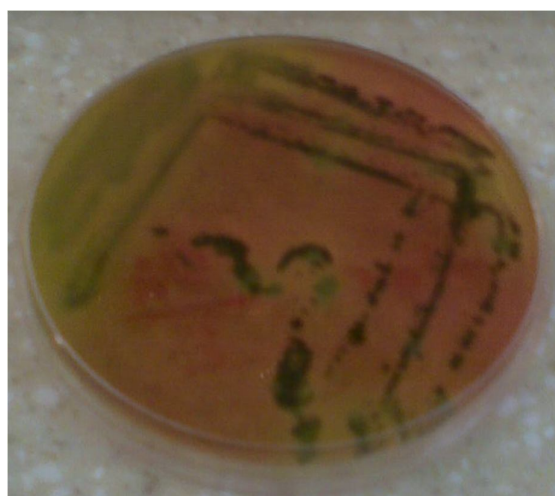
3. Immunohistochemical assays:

Immunohistochemistry was performed as previously described (Hennessy, A.; Pilmore, H. L.; Simmons, L.A. and Paniter , D.M., (1999) for confirmation of the IL-1 and IL-8 in trophoblasts. Briefly, sections (4 µm) were deparaffinized, hydrated, and incubated in 4% hydrogen peroxide in phosphate-buffered saline (PBS) (0.01 M, pH 7.2) for 30 min, incubated with skim milk (1:10 dilution) as a blocking solution for 3

Min. Then incubated with a monoclonal anti IL-1and IL-8 antibody (1:100 dilution) as the primary antibody for 30 min in a humidified chamber at room temperature. After being washed in PBS, the slides were incubated with biotinylated secondary antibody for 20 min at room temperature, washed in PBS again, and then incubated with streptavidin-peroxidase complex (DAKO Corporation, UK) for 20 min at room temperature. The reaction was developed with a 0.024% diaminobenzidine solution (Dako), and sections were counterstained with hematoxylin . Cells that expres of IL-1and IL-8 was detected as brown –black staining. It was graded as 3 ,(75-100%); 2 ,(25-75%) and 1, (< 25%)of the positive epithelial cells for IL-1and IL-8 (8) brown-stained cells and regions were manually counted in ten fields of view from a single placentome. The mean of staine percentage area were used for statistical analysis (Chi-Square).

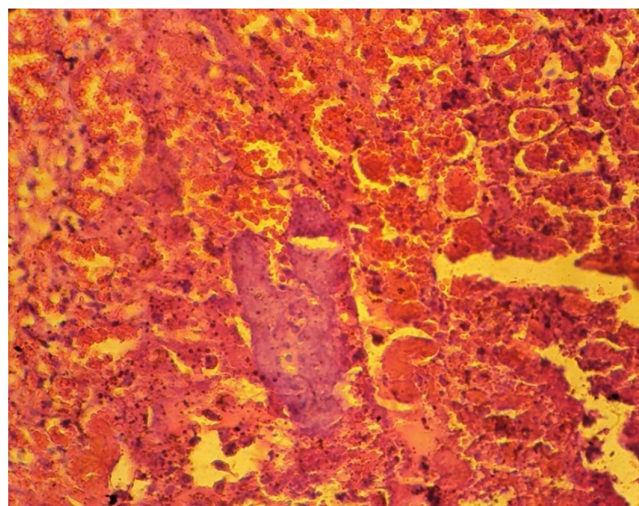
Results:

Twelve placenta samples out of 120 were positive for Salmonella culture which yielded almost pure growth as a yellow smooth colonies on macConky agar, with black sediments on S.S.agar and rabbit like colonies on XLD agar with red colonies on brillent green agar, All isolates were Gram-negative, motile, short rods, oxidase-negative with a typical red slope/yellow butt reaction in triple sugar iron (alkaline slope/acid butt) with the production of high amounts of hydrogen sulphide and gas.(figure1)



Figure(1) shows colonies of Salmonella spp on XLD and producing of H2S.

Histopathologically positive aborted placenta for Salmonellosis showed a necrotizing placentitis (figure: 2). Placentitis were necro-purulent, and necro-hemorrhagic,depending on presence of amorphous materials, infiltration of inflammatory cells including neutrophils and macrophages and exudates.



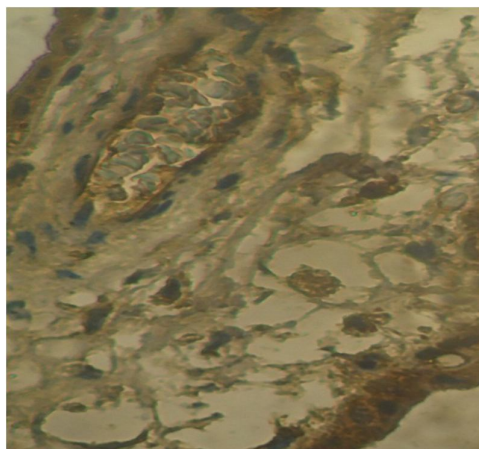
Figure(2): Bovine placental tissue, there are hemorrhagic necrosis(HN) with infiltration of inflammatory cells, neutrophils (Ne), macrophages(M)and accumulation of amorphous material(AM). H&E, 100X.

Immunohistochemical analysis of placental samples showed Positive staining for IL-1 and IL-8 localized in the trophoblast cells in Salmonella positive samples and Salmonella negative samples . (Figure 2,3 and 4).

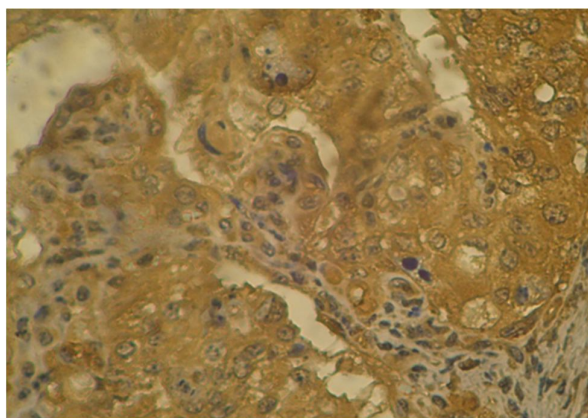
However, the staining intensity for these subjects was much weaker than the immunoreactions seen in the Salmonella positive subjects. The immune staining of IL-1 and IL-8 were positive at high level in Salmonella positive samples were 83.3% (10 out of 12) for IL-1, 91.6%(11 out of 12) for IL-8 .there is significant differences between the infected and uninfected groups ($p>0.05$) table(1).

Table(1). presence of IL-1 and IL 8- in placental cells of aborted cows (IHC assay):

Variable	score	Positive for Salmonellosis		Negative for Salmonellosis	
		NO	%	NO	%
IL-1	1	0	0	9	75
	2	2	16.6	3	25
	3	10	83.3	0	0
IL-8	1	0	0	8	66.6
	2	1	8.3	4	33.3
	3	11	91.6	0	0



Figure(3): Immunohistochemical staining (IHC) of IL-1 in cow placenta infected with *Salmonella*, The sections were stained by DAB chromogen (brown) and counterstained with Hematoxylin (blue) , there is high intensity of IHC reaction. 400X



Figure(4): Immunohistochemical staining (IHC) of IL-8 in cow placenta infected with *Salmonella* The sections were stained by DAB chromogen (brown) and counterstained with Hematoxylin (blue) , there is high intensity of IHC reaction. 400X

DISCUSSION:

Results showed only 12 cases were positive for *Salmonella* culture, this results agree with that reported by (Oliveira, C.J.B.; Carvalho, L.F.O.S.; Fernandes, S.A.; Tavechio, A.T.; Domingues, F.J.Jr. (2005) , culture characteristic of *Salmonella* same with that reported by (Quinn, P.T.; Carter, M.E.; Markey, B. and Carter, G.R. (2004) . Histopathological examination of aborted placenta showed necrosis and placentitis this finding was obtained by (Salcedo, S.P.; Moursadeghi , J.; Cohen , D.W. (2001) who showed that *Salmonella* causes severplacentitis, necroperulant inflammation, hemorrhage and infiltration of inflammatory cells, neutrophils and macrophages and accumulation of amorphous material.

Immunohistochemical assays showed high expression of inflammatory cytokines(IL-1 and IL-8), Several interleukins cess by changing the native Th0

response to a Th1, Th2, or a mixed Th1 and Th2 response (Wick , C.; Hermeling, S.; Bouma,B.; Jiskoot,W. (2003). It was found in biopsy samples with positive MNCs, suggesting a local entrapment of the cytokine in Salmonella infected cells (Uchiya, K.; Barbieri, M.A.; Funato, K. (1999) . Results showed increased levels of the proinflammatory cytokine (IL-1) and (IL-8). The numbers of MNCs and PMN cells staining speand chemokines are involved in the progress of the inflammatory procifically IL-1 and IL-8, in Salmonella positive samples (P<0.05) .Many studies have shown that Salmonella infection is associated with increased IL-1 production within the placenta Moreover, IL-1, a proinflammatory cytokine, is an important intermediary in the resolution of inflammation. It supports transition between the early, predominantly neutrophilic stage of an infection and the more sustained mononuclear cell influx (Raupach, B. and Kaufmann, S.H. (2001).

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