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# Detection of interleukin-6, interleukin-8 and gamma interferon in placentafrom aborted ewes infected with*Listeria monocytogenes*

# التحري عن الانترفيرون نوع غاما والانترلوكين 6و8 في عينات المشيمة الماخوذة من النعاج المجهضة

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## Abstract

Hundred and ten aborted placentas were obtained from ewes and cultured directly,while other were fixed in formalinbuffer to study the effect of interlukin-6, interlukin-8 and gamma-IFNin pathogenesis of *Listeria monocytogenes* associated with placentitis in aborted ewes. Results showed eleven placenta samples from out 110 were positive for listeria culture,wereshowed high expression of IL-6, IL-8 and γ-IFNin aborted placenta infected with *Listeria monocytogenes* as a compared with non infected placentas.

**الخلاصة:**

تم جمع 110عينة مشيمة من نعاج مجهضة , زرعت العينات مباشرة وثبتت باستعمال محلول الفورمالين لمعرفة تاثير كل من الانترفيرون نوع غاما والانترلوكين 8و6على امراضية جرثومة اللستيريا المصاحبة لالتهاب المشيمة في النعاج المجهضة . اظهرت النتائج بان 11عزلة تم الحصول عليها من مجموع النعاج المجهضة وان هناك كان تعبير عالي لكل من الانترفيرون نوع غاما والانترلوكين 8و6 في مشائم النعاج المجهضة و المصابة باللستيريا مقارنة بغير المصابة باللستيريا.

## Introduction

Listeriosis is a food-borne disease caused by *Listeria monocytogenes.* This bacterium is ubiquitous and found throughout the environment including soil, water and decaying vegetation. Substantial proportion of the sporadic cases of listeriosis are caused by consumption of the organism in foods(1). Disease usually occurs in well-defined high risk groups, including pregnant women, neonates and immunocompromised adults, but may occasionally occur in persons who have no predisposing underlying condition(2). Numerous animal species are susceptible to listerial infection, with large proportion of healthy asymptomatic animals shedding *L. monocytogenes* in their feces. Although most infections are subclinical, listeriosis in animals occurs either sporadically or as epidemics and often leads to fatal forms of encephalitis(3). Virtually all domestic animals are susceptible to listeriosis ,animal listeriosis is frequently associated with late term abortions, placentitis, gastrointestinal septicemia with hepatitis, splenitis, pneumonitis and encephalitis. Abortion in sheep due to listeriosis is known to be an important problem in many sheep-raising areas of the world (4).Listeriosis is an irregular disease of some animal species and humans, but it is most significant economically in animals. Furthermore, listeriosis is one of the most frequent causes of meningitis in humans and nonhuman primates (5).The goal of this study was to evaluate the profile of cytokines responses in pathogenesis of placenta infected with *Listeria monocytogenes*

## Materials and methods:

### 1.Aborted ewes:

Hundred and ten aborted ewes were used in this study, where aborted at late stage of pregnancy

### 2.Placenta specimens:

Placentas specimens were obtained from aborted ewes divided intotwo parts, one part cultured directlyon 7% sheep blood agar (Difico), Listeria selective medium (Oxoid), and Eosin Methylene Blue (EMB) agar. The cultures were incubated at 37 C for 3 days, aerobically .Other part of placenta tissue were fixed in 10% buffered formalin, processed routinely, and stained with haematoxylin and eosin (H&E).

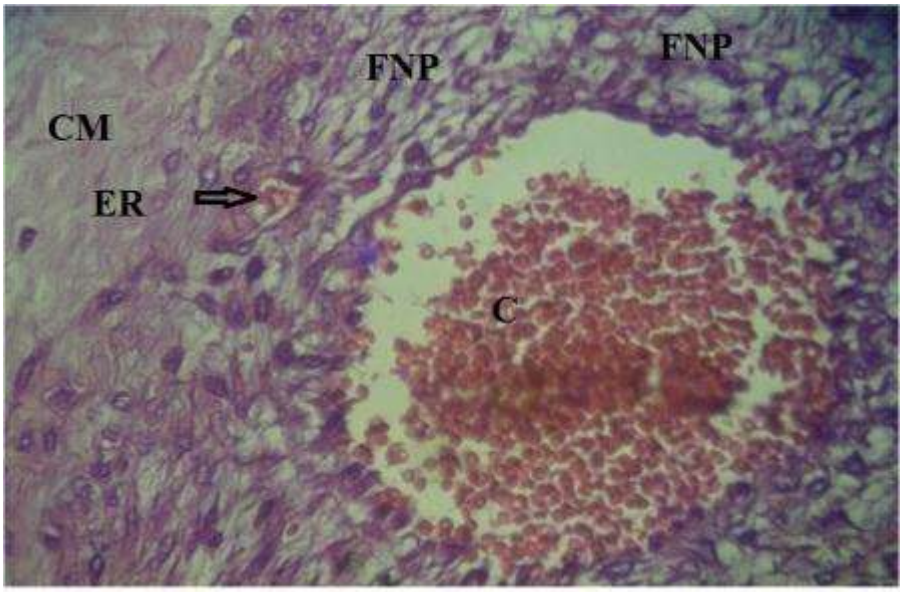
### 3. Immunohistochemical assays:

Immunohistochemistry was performed as previously described (6) for confirmation of the IL-6, IL-8 and γ-IFNintrophoblasts. ( streptavidin-peroxidase complex (DAKOCorporation, UK).
The positivity of cells for expression of IL-6, IL-8 and IFN-γ was seen as brown –black staining(40x). It was graded as 3 ,( 75-100%); 2 ,( 25-75%) or 1, ( < 25% )of the epithelial cells staining positive for IL-6 or IFN-γ (5). brown-stained cells and regions were manually counted in ten fields of view from a single placentome.
Themean percentage area stained values were used forstatistical analysis (Chi-Square).

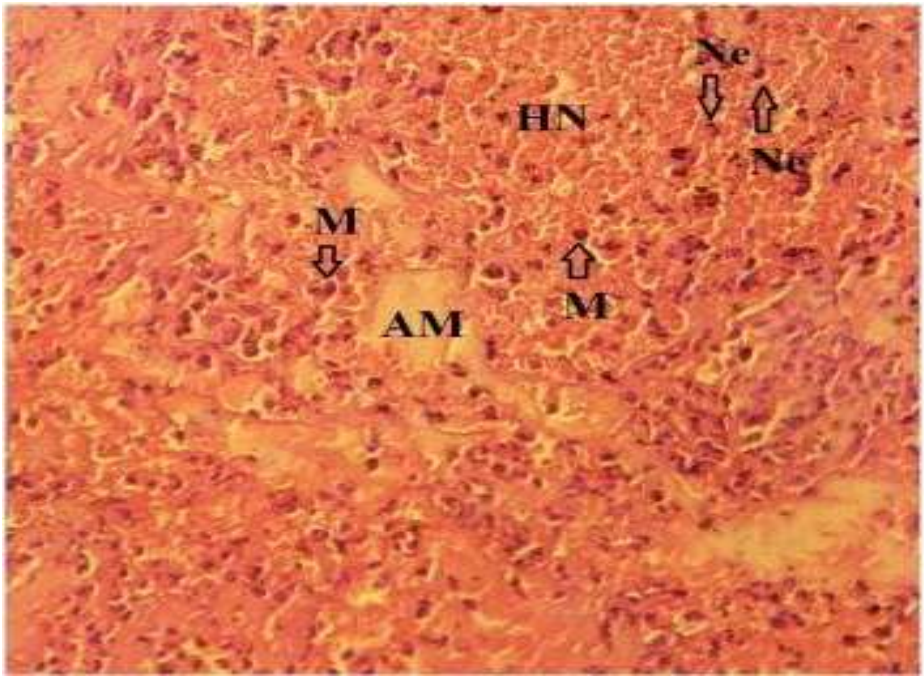
## Results:

Eleven placenta samples from out 110 were positive for listeria culture and yielded almost pure growth of β- haemolytic colonies on the blood agar at the end of the incubation period. The bacterial colonies were small (1-2 mm in diameter), smooth, circular and whitish-grey. On the Listeria selective agar, the colonies had similar characteristics and were 3-4 mm in size. Growth was not observed on EMB agar. Staining of smears from the colonies revealed small Gram-positive bacilli. The isolate was catalase positive, phosphatase, methyl red and Voges-Proskauer testpositive, it hydrolysed aesculin and exhibited tumbling motility between 25 and 30 C. The CAMP test was positive with *S. aureus*.

Aborted placenta positive for listeriosis histopathologically showed,the necrotizing placentitis was seen(figure:1, 2). Placentitis were classified respectively as necro-purulent, and necro-hemorrhagic, depending on the presence of amorphous materials, infiltration of inflammatory cells including neutrophils and macrophages and exudates.




**Figure(1): Fibrinoid necrotic placentitis(FNB) of chorionic membrane with congestion of blood vessels(C ) and extravasated RBCs(ER). H&E, 400X.**



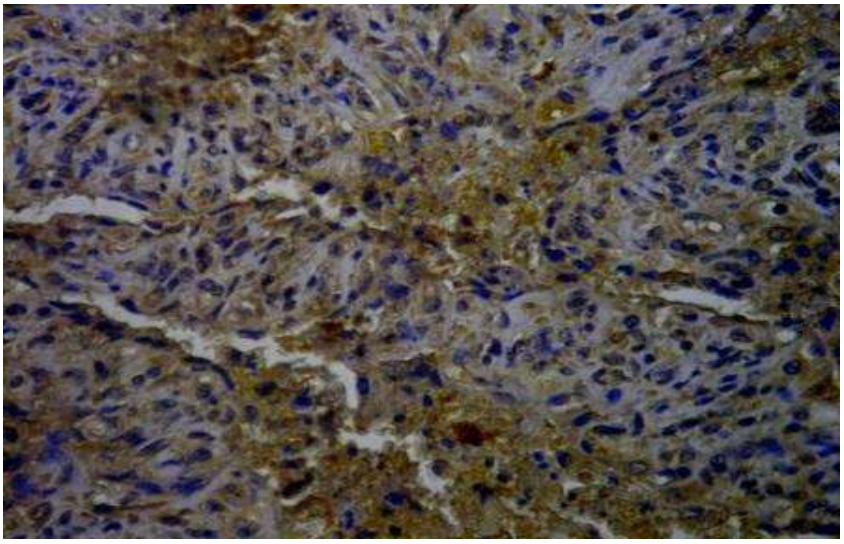

**Figure(2): Ovine placental tissue, there are hemorrhagic necrosis(HN) with infiltration of inflammatory cells, neutrophils (Ne) and macrophages(M)and accumulation of amorphous material(AM). H&E, 100X.**

Immunohistochemical analysis of placental samples showed Positive placental staining was seen forIL-6, IL-8 and γ-IFNlocalized to the trophoblast cells (Fig.3,4 and 5)in aborted ewes positive for listeriosis, Also positive placental staining forIL-6, IL-8 and γ-IFNwas seen in the*listeria*non infectedsubjectsHowever, the staining intensity for these subjects was much weaker than the immunoreactions seen in the*listeria*postivesubjects. The immune staining of IL-6, IL-8 and γ-IFNwere positive at high level in aborted postive for listeriosiswere81.8% (9 out of 11) for IL-6, 90.9%(10 out of 11) for IL-8 and 90.9%(10 out of 11) for γ-IFN.there is significant differences between the infected and uninfected groups ($p$>0.05) table(1).

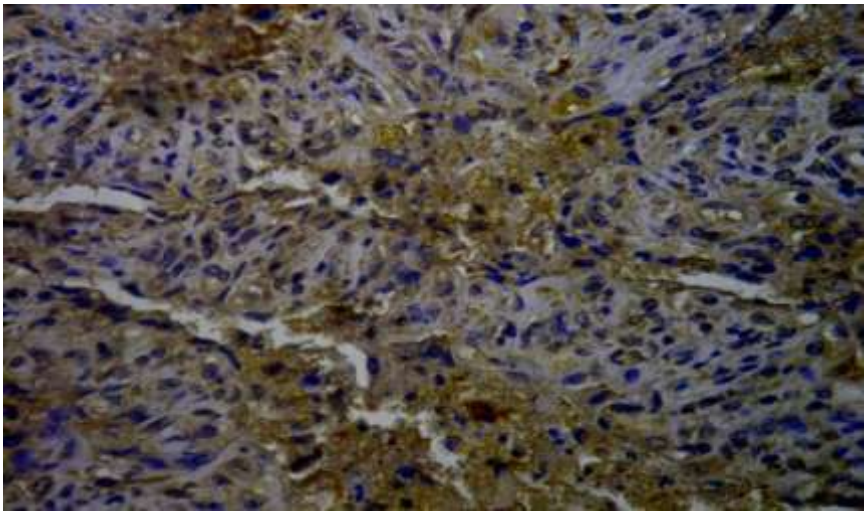
Table(1). presence ofIL-6, IL-8 and γ-IFN in placental cells of aborted ewes (IHC assay):

| variable | score | Positive for listeriosis | | Negative for listeriosis | |
|---|---|---|---|---|---|
| | | NO | % | NO | % |
| IL-6 | 1 | 0 | 0 | 8 | 72.7 |
| | 2 | 2 | 18.1 | 3 | 27.2 |
| | 3 | 9 | 81.8* | 0 | 0 |
| IL-8 | 1 | 0 | 0 | 7 | 63.6 |
| | 2 | 1 | 9 | 4 | 36.3 |
| | 3 | 10 | 90.9* | 0 | 0 |
| γ-IFN | 1 | 0 | 0 | 8 | 72.7 |
| | 2 | 1 | 9 | 3 | 27.2 |
| | 3 | 10 | 90.9* | 0 | 0 |

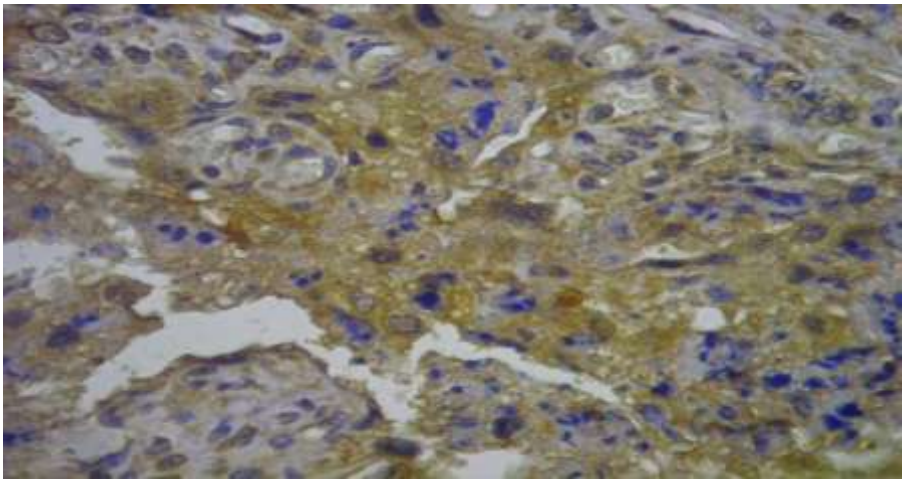
(*= significant)



**Figure(3): Immunohistochemical staining (IHC) of IL-8 in ewe placenta infected with *listeria*, 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-6 in ewe placenta infected with *listeria*, The sections were stained by DAB chromogen (brown) and counterstained with Hematoxylin (blue) , there is high intensity of IHC reaction. 400X**



**Figure(17): Immunohistochemical staining (IHC) of γ-IFN in ewe placenta infected with *listeria*, The sections were stained by DAB chromogen (brown) and counterstained with Hematoxylin (blue) , there is high intensity of IHC reaction. 400X**

**DISCUSSION:**

From results showed only(11)cases were positive for *Listeria monocytogenes*, the culture characteristic of *Listeria* same with that reported by(7).Histopathological examination of aborted placenta showed necrosis and placentitis this finding same to that obtained by(8)who showed that listeria causes sever placentitis,necroperulant inflammation, hemorrhage and infiltration of inflammatory cells, neutrophils and macrophagesand accumulation of amorphous material.

Immunohistochemical assays showed high expression of inflammatory cytokines(IL-6and IL-8) and CMI cytokines (γ-IFN),Several interleukins and chemokines are involved in the progress of the inflammatory process by changing the native T- helper 0 response to a T-helper 1, T-helper 2, or a mixed T-helper 1 and T-helper 2 response(9), (10).In the present study, IFN-γ-specific staining the trophoplast cells, was found significantly difference between the infected and uninfected suggested that the epithelial IFN-γ staining probably reflects receptor-bound IFN-γ, since this cytokine is produced only by T cells and NK cells. Furthermore, it was found in biopsy samples with positive MNCs, suggesting a local entrapment of the cytokine in *listeria*infected cells(11), (12).also detect increased levels of the proinflammatory cytokine (IL-6) and (IL-8), and the numbers of MNCs and PMN cells staining specifically IL-6 and IL-8, in biopsy specimens from *listeria*-infected animals with significant difference ($p < 0.05$) between infected and uninfected cases , as previously reported, many studies have shown that *Listeria monocytogenesis*infection is associated with increased IL-6 production within the gastric mucosaMoreover, IL-6, 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 (13).

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