

## **Isolation of bacterial causes of abortion from cows in Karbala province**

### **عزل المسببات البكتيرية للإجهاض من الأبقار في محافظة كربلاء**

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#### **Abstract**

This study was aimed to investigate the bacterial species that responsible for abortion in the caws at Kerbala city .This performed by testing 120 of aborted caws from different regions of Kerbala city through period of 8 months started from May till December 2012.

Clinical signs & clinical examination were used as a parameters in our study that performed on the aborted caws and fetus through last day of gestation , as well as using of blood samples and vaginal discharge for bacterial examination and isolation .

Results showed that *Brucella spp* was the main bacterial cause of abortion in the tested aborted caws and the percentage of infection reach to 13.33% , while other bacterial spp were detection in the present study but in lower percent as *Staphylococcus aureus* (5.83%) , *E. coli* (4.16%) , *Salmonilla spp* (1.66%) ,*Campylobacter fetus* (1.66%) and *Listeria monocytogenes* (0.83%).

#### **المستخلص**

أجريت الدراسة الحالية في عدة مناطق مختلفة من محافظة كربلاء خلال مدة ثمانية شهور من شهر أيار ولغاية كانون الأول لعام 2012 وذلك للتحري عن الجراثيم البكتيرية المسببة للإجهاض في الأبقار في كربلاء. تم خلالها فحص 120 من الأبقار المجهضة من خلال ملاحظة العلامات السريرية والفحوصات السريرية والمختبرية والتي شملت فحص نماذج الدم والسوائل المهبلية للأبقار المجهضة وذلك لعزل وتشخيص أنواع البكتيريا المسببة للإصابة فضلا عن فحص الأجنة المجهضة أيضا.

تبين من خلال نتائج الدراسة الحالية التي تم التوصل إليها أن نسبة 13.33% من البكتيريا المسببة للمرض يعود إلى نوع *Brucella spp* بالإضافة إلى وجود مسببات بكتيرية أخرى تم الكشف عنها في هذه الدراسة ولكن بنسب إصابة أقل مما ذكر أعلاه وشملت *Staphylococcus aureus* (5.83%) , *E. coli* (4.16%) , *Salmonilla spp* و *Campylobacter fetus* بنسبة (1.66% ) لكلا منهما بينما لوحظ وجود بكتريا *Listeria monocytogenes* وبأقل نسبة إصابة وصلت إلى (0.83% ).

#### **Introduction**

Abortions have a greatly negative impact on reproductive competence, resulting in important economic losses for the cattle industry (1). Under best laboratorial conditions, etiologic diagnosis is reached in 23.3 to 45.5% of the cases (2). Bovine abortion may be due to infectious, toxic, endocrine, physical or nutritional causes. Infectious agents associated with abortion in cattle include viruses, bacteria, protozoa, and fungus. The correct quantity of cases due to infectious agents is not known, but in 90% of cases in which an etiologic diagnosis is achieved the cause is infectious (3). Efficient diagnosis requires a complete diagnostic protocol associated with submission of appropriate specimens and clinical history. conventional diagnostic apparatus include serology, histopathology, bacterial and viral isolation, and for certain agents direct examination or immunohistochemistry, identification of infectious agents in aborted bovine fetuses, including *Brucella abortus*, *Leptospira* sp, *Listeria monocytogenes*, *Campylobacter fetus* subsp. *Venerealis*, *Neospora caninum*, *Mycoplasma bovis*, *Mycoplasma bovigenitalium* , *Chlamydomphila abortus* , *Salmonella enterica* ser. Dublin or *Salmonella* sp. *Arcanobacterium pyogenes* With the exception of *Arcanobaterium pyogenes* and *Salmonella enteric* ser. Dublin, these agents are primarily involved in reproductive diseases in cattle. Although *A. pyogenes* is not a specific cause of abortion, it may cause a suppurative placentitis and abortion Similarly, *Salmonella dublin* is 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 (4).

**The aim of this study is to investigation and detection of the most common species of bacteria that responsible for causing abortion in caws at Kerbala city.**

## **Materials and Methods**

### **Materials:**

#### **1.culture media.**

**Table -1- the substances that used in the preparation of bacteria culture.**

<b>No</b>	<b>Culture media</b>	<b>Company / origin</b>
<b>1</b>	Blood Agar Base	Oxoid / UK
<b>2</b>	<i>Brucella</i> Agar	Oxoid / UK
<b>3</b>	MacConky agar	Fluka / India
<b>4</b>	Nitrate Water	Oxoid / UK
<b>5</b>	Peptone water	Oxoid / UK
<b>6</b>	Trypticase Soya Broth	BBL / USA
<b>7</b>	Trypticase Soya Agar	BBL / USA
<b>8</b>	Urea Agar	Difco / USA
<b>9</b>	Gelatin Agar	Difco/ USA
<b>10</b>	Simmon's Citrate	Himedia/India
<b>11</b>	MR – VP Broth	Difco / USA
<b>12</b>	Manitol salt agar	Difco / USA
<b>13</b>	TSI	Difco / USA
<b>14</b>	Blooa azid agar base	Difco / USA

2. Gram's stain solution and Modified Ziehl – Neelsen stain solution prepared and used as described by Alton *et al* .,(5)

3. Rose Bengal antigen: provided by Omega company, UK.

### **Methods:**

1. Clinical observations: Clinical examination has been performed on aborted cows and fetuses throughout the last few days of gestation period. At parturition, retained placenta, body temperature and bleeding status has been registered .

2. Blood samples:

One Hundred - twenty blood samples were obtained via jugular vein during the period extended from May, 2012 to December, 2012, at Kerbala city . serum samples have been obtained for Rose Bengal test.

3. Aborted fetuses and vaginal discharge.

Fifty aborted fetuses and 70 vaginal discharge swabs, at the first and last stage of pregnancy The specimens were cultured in duplicated blood agar plates as described by Quinne *et al* (6), then purification of isolates on selective media(macconky agar, manitol salt agar, blood azid agar,

brucella selective media, listeria selective media), after that determine all isolates biochemically with biochemical tests.

### **Results**

Twenty of 120 blood samples were positive for RB test . six of 50 aborted fetuses were positive for *Brucella* culture and 10 of 70 vaginal swabs were positive for *Brucella* culture, all cases of brucellosis were found in late stage of pregnancy (5 – 9 month), some cases accompanied with abortion with retention of placenta, Pneumonia and pericarditis were the main complications shown in aborted fetus positive for brucellosis. Placentas were, edematous and opaque with bleeding in some of cases (Figure 1), *Brucella* organisms first recognized in smears obtained from fetal stomach stained with modified ziehl neelsen stain (figure 2), which appeared red clumps against a blue background, *Brucella* culture recognized on the basis of colonial morphology which appeared round translucent pale honey color. One isolate of *Listeria monocytogenes* was obtained from aborted fetuses, *Listeria monocytogenes* culture recognized on the basis of colonial morphology which appeared round translucent, bluish color when viewed from above and produce  $\beta$ -hemolytic on blood agar. Two isolates of *Campylobacter fetus* were obtained from aborted fetuses, placenta was ticking and contain gelatinous exudates. Ten fetus don't produce growth of bacteria on culture media other fetuses were highly contaminated cause emphysematous. Two isolates of *Salmonella* spp were obtained from vaginal discharge were non fermentable on macconky agar and produce H<sub>2</sub>S on S.S. agar , 5 isolates of E.coli and 7 isolates of Staphylococcus aureus were obtained from vaginal discharge. (table 2)

**Table 2-Species and the percent of bacteria that responsible for abortion in the tested aborted cows.**

<b>Species of Isolated bacteria</b>	<b>No</b>	<b>%</b>	<b>Pregnancy period</b>
<i>Brucella ssp</i>	<b>16</b>	<b>13.33</b>	<b>Late stage</b>
<i>Staphylococcus aureus</i>	<b>7</b>	<b>5.83</b>	<b>Different in late and in first</b>
<i>E.coli</i>	<b>5</b>	<b>4.16</b>	<b>Different in late and in first</b>
<i>Salmonella spp</i>	<b>2</b>	<b>1.66</b>	<b>In second stage</b>
<i>campylobacter fetus</i>	<b>2</b>	<b>1.66</b>	<b>In second stage</b>
<i>Listeria monocytogenes</i>	<b>1</b>	<b>0.83</b>	<b>Late stage</b>
<b>Total no. (120)</b>	<b>33</b>	<b>27.5</b>	



Figure (1). Aborted fetus due to brucellosis.

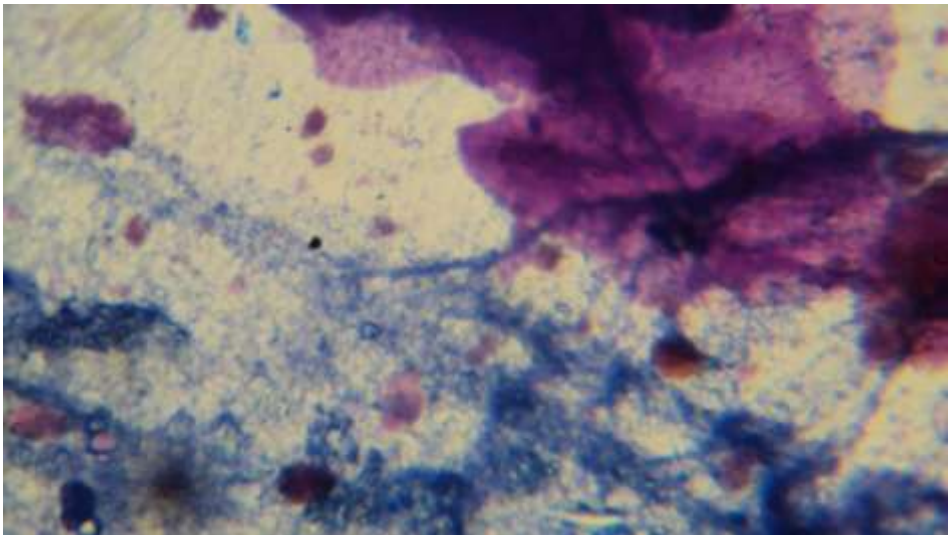


Figure (2). Modified ziehl neelsen stain of aborted fetal stomach

**DISCUSSION:**

*Brucella spp* was the most frequent agent identified with an overall frequency of 13.33%. These results support the notion that *B. abortus* is the most prevalent cause of infectious abortion in Kerbala province which is in good agreement with (7) all aborted fetuses infected with *Brucella* showed pericarditis and pneumonia this same that reported by (8). From results of present study showed *S. aureus* also causes abortion in cows may be due to this bacteria contains hydrolysis enzyme and toxins that may affect the fetus and placenta leading to abortion (9), *E.coli* also caused abortion in cow as in table (1) result may be from endotoxine effect of LPS of bacteria . in the present case the endocarditis cannot be attributed to *L. monocytogenes* with certainty since no organisms were observed in association with the lesion, which is often the case in *L. monocytogenes* induced lesions(10). Infection with *L. ivanovii* is recognized as a cause of abortions and stillbirths in cows (11), as well as of the birth of live lambs that are weak and often fail to survive, the abortions occurred the weather had been . extremely cold and wet because of heavy rainfall. A change to cold and wet weather and the confinement of sheep in sheds have been reported to favour the growth and spread of Listeria micro-organisms(12).

*Campylobacter* also obtained in this study but was rare as compared with *Brucella spp* infection and agree with(13) . *Salmonella spp* cause abortion may be due to fever, this same results reported by (14).

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