



Relationship between infection with Campylobacteriosis and genital disorder in ewes

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Abstract

Bacteria is the main cause of the genital disorder. Abortion, recurrent abortion and placenta retention are the main disorder accompanying with pregnancy failure. The current study aimed to find the relationship between this disorder and Campylobacter infection and main Campylobacter species related to this disorder. For this purpose, 50 aborted fetuses and vaginal swabs were collected. Case history and multiplex PCR were applied. The result showed that Campylobacter detected at a rate 26% in abortion cases and 61.5% of positive cases of campylobacter spp. are accompanied by placenta retention. The current study revealed that 75% of positive cases of campylobacter spp. accompany with recurrent abortion. According to MPCR test, Campylobacter fetus, Campylobacter jejuni, and Campylobacter coli were detected in a rate of 86.65%, 12.3%, and 0%, respectively.

Key words: Campylobacteriosis, genital disorder, abortion

Introduction

Many bacterial causes abortion in sheep. *Campylobacter* spp. one of them and may occur asymptomatic or cause abortions and infertility. *Campylobacter* spp. may also cause enteritis and ileitis (1). The main pathogenic species of *Campylobacter* are *Campylobacter fetus* subsp. *fetus* and *Campylobacter jejuni* which cause abortion in sheep. Thermophilic campylobacters (*C. coli*) may also cause abortions in sheep (2).

The first isolation of *Campylobacter* from aborted sheep fetuses was in 1909. While, it was named in 1963. *Campylobacter* is gram negative bacteria, curve, helical or gull-shape. It shedding throw feces, aborted fetuses' placentas and uterine discharges, these sources may be contamination environment (3). *Campylobacter* transmission by ingestion of contaminated feed, water aborted material and via sexual transmission (4,5). The gross lesion appear on aborted or stillborn fetuses are subcutaneous edema, necrotic liver lesions or bronchopneumonia. On enlargement placental, cotyledons are covered with blood-tinged exudate revise. Microscopically main lesion appear on placenta are predominant in hilar zones, arterioles, leukocyte infiltration and accumulation of high numbers of bacteria within leukocyte (2,4).

Material and methods

Sampling: The current study conducted on Salahaldeen province in period from January/2021 to December/2021. Fifty abortion ewe's cases were subjected in age 3-5 years, case history was taken and clinical examination preformed.

Twenty aborted fetus (abomasum content) and 30 vaginal swabs were collected in a

period not more than 7 days. The samples were directed culturing in Brain-hart infusion broth (Himedia-India) and cultivated at 37°C for 48h.

DNA extraction: By use of ABIOPure™ Total DNA (ABIOPure, USA) according to Manufacturer's instructions. Primer used: three primers were used in the current study

a. *Campylobacter fetus* (give DNA product in size 143bp)

F-5'-GCCAAGCTCATTTGCAGAGT-3'

R-5'-ACGCCAAAGACGGTGTGAG-3'

b. *Campylobacter jejuni* (give DNA product in size 364bp)

F- 5'-TGGAAGTGCTCCAGAAAAGG-3'

R- 5'-GCCAAATAATTCTTTTGCAAC-3'

c. *Campylobacter coli* (give DNA product in size 502bp)

F- 5'-GGT ATG ATT TCT ACA AAG CGA G-3

R- 5'-ATA AAA GAC TAT CGT CGC GTG-3

DNA reaction mixture: 25µM consist from: 3µM of DNA template, 12µM Master Mix, 1µM from each forward and reverse primers, and 4µM Nuclease Free Water.

Thermocycler programs: consist of: Initial Denaturation at 95°C for 5 min, then 30 cycles, each cycle consists of Denaturation at 95°C for 30 sec, Annealing: at 60°C for 60 sec and Extension: at 72°C for 60 sec. The final Extension: at 72°C for 7 min. Then hold at 10°C for 10 min.

Agarose Gel Electrophoresis: the electrophoresis done on agarose gel (2%) and

Electrical power was turned on at 100v/mAmp for 75 min.

Results

The present study showed that out of 50 abortion cases, *Campylobacter* was detected in 13 cases with rate of 26% (Table 1). The current study demonstrated that 61.5% of positive cases of *campylobacter* spp. is accompany with placenta retention (Table 2). On the other hand, 75% of positive cases of *Campylobacter* spp. is a accompany with recurrent abortion that's mean either the *Campylobacter* infection not produce effective immunity level or may other infection due to previous abortion (Table 3) The current study revealed that all the 13 detected cases were belong to species *C. fetus*, 2 cases were diagnosed as *C. jejuni*, and these two isolates detected as mix infection with *Campylobacter fetus* (Figure1 and 2). While, not detected any isolate belongs to *C. Coli* (Table 4).

Discussion

About 80% of abortions in ewes caused by *Brucella*, *Toxoplasma*, *Campylobacter*, and *listeria*. *C. fetus* and *C. jejuni* is the main *Campylobacter* species associated with sheep abortion. All the aborted ewes and fetus should disengage in the field because they are a source of the spreading the infection (6). In the current study, *Campylobacter* caused abortion in a rate of 26%. This finding is in agreement with previous study in Suleimani province which is 26.8% (29 out of 108) (7). While, this rate is high than the rate recorded in Sistan region which is 7% (8). in Uruguay, *Campylobacter* recorded as abortion cause in a rate of 6% (9). The detected rate is less than the rate that recorded in Iran which is 50.6% (10). In the current study, the difference between

infection recoded in compare with other study may due to geographic differences in the study area, seasonal of the study, techniques used in diagnosis, clinical signs management and control programs (11). The current study showed that high relationship between *Campylobacter* infection and placenta retention, that's due to the ability of *Campylobacter* to cause placentitis and the cotyledons separate easily from the caruncles. In addition, the placentitis lead to fibroblast activation which produce fibrin that leads to the attachment of placenta on uterus (12,13). The present study showed that 75% of abortion cases diagnosis as *campylobacter* infection were suffering from recurrent abortion, that's may due to other causes of previous abortion such as *Brucella*, *Salmonella*, *Toxoplasma* and non-infection causes or due to expos to high dosage of bacteria by contact with aborted fetus, placenta, and uterine discharges (14, 15). *Campylobacter* spp. are common abortion causes in ewes, most abortion cases occur at the twelfth week of pregnancy and after 7-25 days after infection (17).

In the current study, *C. coli* was not detected. This finding is in agreement with previous studies (7,8,9,10). *C. Coli* is related to digestive system infection and is not highly related to abortion (18). In a study conducted in Baghdad, four *Campylobacter* spp. were isolated which were *C. jejuni*, *C. coli*, *C. lari* and *C. fetus* which from diarrheic and non-diarrheic cows (19). In the current study *C. fetus* is the main species of *Campylobacter* related to abortion in ewes. This finding is in agreement with the results of previous studies (7,8,9, and 10). *C. fetus* is the main cause of abortion in sheep. *C. fetus* expresses have a Para crystalline surface layer (S-layer) which makes bacteria resistant to serum killing by impairing C3b binding

(20). Pathogenesis of *C. fetus* includes bacteremia, placentitis, uterine, and fetal infection, the fetus suffering from 1–2 cm orange/yellow necrotic foci in the liver. Placentitis with hemorrhagic, necrotic cotyledons and edematous or leathery areas between the cotyledons (21, 22).

Conclusion: highly relationship between Campylobacteriosis with abortion, recurrent abortion, and placenta retention. *C. fetus* is the main species related to genital disorder in ewes.

Table 1: Number and Rate of positive cases of *Campylobacter* spp.

Sample type	No of positive cases	Rate of positive cases (%)
Aborted fetus (20)	6	30
Vaginal swabs (30)	7	23.3
Total (50)	13	26

Table 2: The relationship between placenta retention and *Campylobacter* infection.

Placenta state	No of positive cases	Rate of positive cases (%)
Aborted with placenta retention (17)	8	61.5
Aborted with placenta retention (33)	5	15.1
Total (50)	13	26

Table 3. The relationship between recurrent abortion and *Campylobacter* infection.

Case history	No of positive cases	Rate of positive cases (%)
recurrent abortion (8)	6	75%
First abortion (42)	7	16.6%
Total (50)	13	26%

Table 4: *Campylobacter* species detected in the current study.

Campylobacter species	No of positive cases	Rate of positive cases (%)
<i>Campylobacter fetus</i>	13	86.6%
<i>Campylobacter jejuni</i>	2	12.3%
<i>Campylobacter Coli</i>	0	0%
Total	15	100%

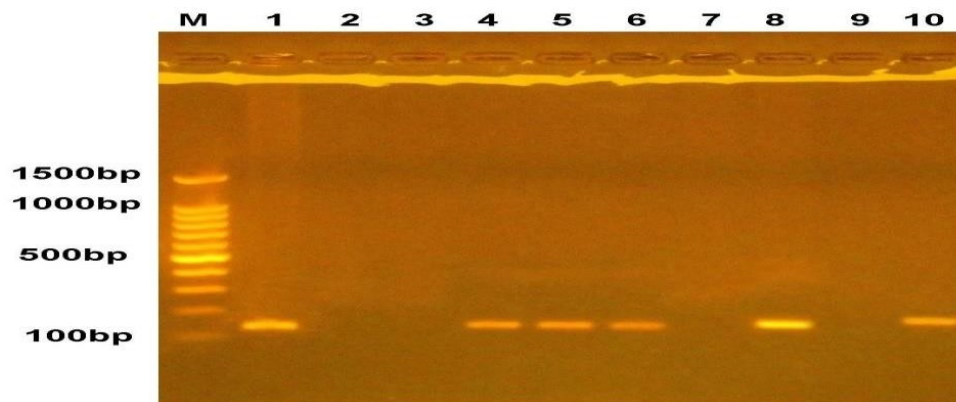


Figure 1. Agarose gel electrophoresis of MPCR for detection of *Campylobacter*spp. M: 100bp DNA marker, lens 1,4,5,6,8,10 positive to *Campylobacter fetus* which give band in size 143bp.

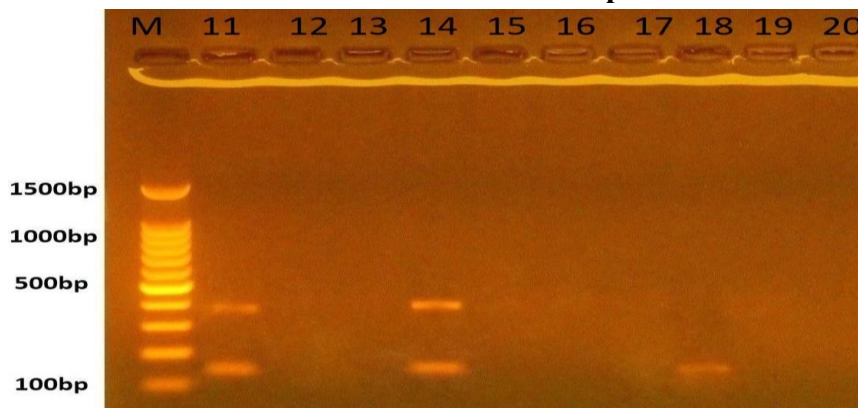


Figure 2. Agarose gel electrophoresis of MPCR for detection of *Campylobacter* spp. M: 100bp DNA marker, 18 positives to *Campylobacter fetus* which give band in size 143bp. Lens 11&14 mix infection with *Campylobacter fetus* which give band in size 143bp and *Campylobacter jejuni* give band in size 364bp.

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العلاقة بين الإصابة بداء العطائف واضطراب الأعضاء التناسلية في النعاج

الخلاصة

الجراثيم هي السبب الرئيس لإصابات الجهاز التناسلي. الإجهاض والإجهاض المتكرر واحتباس المشيمة هي الأسباب الرئيسة والمصاحبة لفشل الحمل. تهدف الدراسة الحالية إلى إيجاد العلاقة بين الإجهاض وعدوى جراثيم *Campylobacter* مع حدوث هذه الاضطرابات وكذلك علاقة أنواع جراثيم *Campylobacter* الرئيسة مع هذه الاضطرابات. لهذا الغرض تم جمع 50 جنيناً مجهضاً ومسحة مهبلية. تم اخذ تاريخ الحالة وجراء فحس تفاعل البلمرة المتسلسل PCR المتعدد. أظهرت النتائج أن *Campylobacter* اكتشفت بمعدل 26% في حالات الإجهاض، و61.5% من الحالات الإيجابية *Campylobacter* كانت مصحوبة باحتباس المشيمة. كما كشفت الدراسة الحالية أن 75% من الحالات الإيجابية للجراثيم *Campylobacter* كانت مصاحبة للإجهاض المتكرر. وفقاً لاختبار MPCR، تم اكتشاف *Campylobacter fetus*، *Campylobacter jejuni*، *Campylobacter coli* بمعدل 86.65% و 12.3% و 0% على التوالي.