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Use of Multiplex PCR for Detection of *Campylobacter* spp. and their Virulence Factors in Aborted Ewes

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Abstract | The current study aimed to detect and map virulent factors of Campylobacter spp. from aborted ewes. A total of 80 samples were collected from aborted ewes (28 from stomach contain of aborted fetus and 52 vaginal swabs collected from aborted ewes in period not more than 48 hours after abortion). Total, DNA extraction followed by multiplex PCR showed that *Campylobacter fetus* infection rate was 13.7% (11:80) while Campylobacter Jejuni was detected in 3.7% (3:80). Additionally, detection of *cadF, cdtB* and *cdtC* gene was confirmed in 100%, 78.5% and 85.7%, respectively while Iam1 gene was detected with rate of 7.1%. Taken together, *Campylobacter* spp. is an important cause of abortion in ewes and *Campylobacter* adherence gene (*CadF*) was detected the most frequent gene detection. Campylobacteriosis is the leading cause of ewe abortion in the area. C. fetal is the most pathogenic *Campylobacter* species in our region, affecting 17.5% of ewes.

Keywords | Campylobacter spp., Aborted ewes, Virulence factor

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INTRODUCTION

Campylobacteriosis is a zoonotic disease, infecting sheep, goat, cattle and human. In human, it causes enteritis, while in sheep and cattle causes reproductive disease (Hlashwayo *et al.*, 2020). *Campylobacteriosis* in sheep, goats and cattle may cause enteric diseases, which is characterized by watery or bile-streaked diarrhea or may contain blood or mucoid, vomiting and sometimes fever. Other important disease caused by *Campylobacter* spp. is reproductive disease (Hagos *et al.*, 2021). Campylobacter fetus or *Campylobacter jejuni* cause abortion in the late stage of pregnancy with gross lesions in the placenta and can cause stillbirths and weak lambs, sometime these cases followed by metritis (Mewari and Chauhan, 2020). *Campylobacter* sheds in vaginal discharges, aborted fetuses and fetal membranes of aborting sheep (Souhayla *et al.*, 2017; Büyük *et al.*, 2020). Main pathological changes include subcutaneous edema, and in rarely cases blood-stained fluid showed on body cavities, yellow or dark brown exudate and necrotic plaques may be observed on fetal cotyledons, and oedema may occur in the inter-cotyledonary areas (Abdulameer and Saleem, 2018; Rush and Edmondson, 2021). Campylobacter carry many virulence factors, like cytolethal distending toxin B (cdtB), which broadly spared among gram negative bacteria and

Table 1: Primers used in the current study for detection of *Campylobacter* spp.

Gene name	Sequences		Product size	References
Campylobacter fetus	F	5-GGAAGCCGCAGCTGCTAAGAT-3	359	Persson and Olsen, 2005
	R	5-AGCCAGTAACGCATATTATAGTAG-3		
Campylobacter Jejuni	F	5-CAAATAAAGTTAGAGGTAGAATGT-3	161	
	R	5-CCATAAGCACTAGCTAGCTGA T-3		
Campylobacter adherence gene (*CadF*)	F	TTGAAGGTAATTTAGATATG	400	Yoon, 1999
	R	CTAATACCTAAAGTTGAAAC		
Cytolethal-distending-toxin subunit-B gene (*CdtB*)	F	GTTGGCACTTGGAATTTGCAAGGC	495	Bang *et al.*, 2003
	R	GTTAAAATCCCCTGCTATCAACCA		
Cytolethal-distending-Toxin subunit-C (*CdtC*)	F	CGATGAGTTAAAACAAAAAGATA	182	Rozynek *et al.*, 2005
	R	TTGGCATTATAGAAAATACAGTT		
Invasio´n-associated marker 1 (*Iam1*)	F	GCGCAAAATATTATCACCC	512	Rozynek *et al.*, 2005
	R	TTCACGACTACTATGCGG		

cause damage and subsequent death on membranes of host cell (Shams *et al.*, 2016). Campylobacter has ability to adhere to host epithelial cell, this process encoded by fibronectin F (cadF) and fibronectin is brokered by CadF fibronectin binding outer membrane protein (Konkel *et al.*, 1997), and the heat survival and stress response proteins htrB and clpP, which are important for survival (Elhadidy *et al.*, 2018).

This study aims to identify and characterize *campylobacter species* from aborted ewes and to determine the antibiotic resistance markers for the selection of antibiotic use in livestock.

MATERIALS AND METHODS

A total of 80 samples were collected from aborted ewes (28 from stomach contain of aborted fetus and 52 vaginal swabs collected from aborted ewes in period not more than 48hours after abortion). DNA extraction was using Intron kit on isolate from stomach contain of aborted fetus and vaginal swabs. The PCR amplification was performed using the primers mentioned in Table 1 to detect and confirm *Campylobacter* spp. and virulence factors.

Table 2: Number and ratio of *Campylobacter* spp detected in current study.

Campylobacter spp.	Number of samples	Number of detection case	Rate of detection
Campylobacter fetus	80	11	13.7%
Campylobacter Jejuni		3	3.7%
Total		14	17.5%

RESULTS AND DISCUSSION

Out of 80-abortion sample, *Campylobacter* fetus was detected 13.7% (11/80) of samples while *Campylobacter Jejuni* was detected in 3.7% (3/80) as in Table 2 and Figure 1.

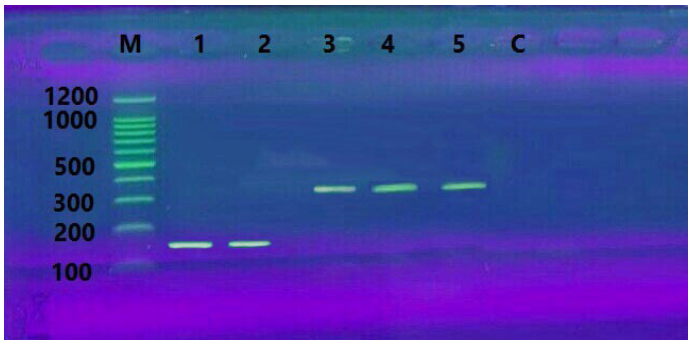

Figure 1: Electrophoresis of multiplex PCR product on agarose gel. M: 100bp DNA marker, lanes 1 and 2: 161bp fragment of *C. Jejuni*. Lanes 3, 4 and 5: 359bp fragment of *C. fetus*

The result of current study differed than result of a previous study (Hamali *et al.*, 2014), where authors have detected of *C. fetus subsp. fetus* and *C. jejuni* with a rate of 9% and 1.5% respectively. In another study, (Rizzo *et al.*, 2015) authors have detected of *campylobacter* spp. with a rate of 3.5%. This difference might be due to difference in the geographical region, number of samples, clinical signs, age and species of animals. The detection of *Campylobacter* spp. in abortion case endorses that *campylobacter* spp. is one of important etiology of abortion in sheep. Many studies (Mannering *et al.*, 2006; Chon *et al.*, 2020; Dorsch *et al.*, 2021; Şevik, 2021), detected *Campylobacter* spp. from abortion ewes. *C. fetus* and *of C. jejuni* caused infertility, early embryonic death and a prolonged lambing season. *C. fetus* caused abortion in the lasted pregnancy period, stillbirths and weak lambs. In some cases, metritis and occasionally deaths may occur (Dawson *et al.*, 2020). Our findings showed that the detection rate of *C. fetus* more than detection *rate of C. jejun,* which agreed with result of another study (Tamura and Kumar, 2004). The application of multiplex PCR for

Table 3: Number and Rate of *Campylobacter* spp isolates possessing virulence factor.

Campylobacter virulence factors	Total isolate	DNA out product size	Number of isolates possessing virulence factor	Rate of isolates possessing virulence factor
Campylobacter adherence gene (*cadF*)	14	400	14	100%
Cytolethal distending toxin subunit B gene (*cdtB*)	14	495	11	78.5%
Cytolethal distending Toxin subunit c (*cdtC*)	14	182	12	85.7%
Invasio´n-associated marker 1 (*Iam1*)	14	512	1	7.1%

the detection of campylobacter virulence revealed that cadF, cdtB and cdtC were detected with a rate of 100%, 78.5% and 85.7% respectively while Iam1 detection rate was 7.1% as in Table 3 and Figure 2.

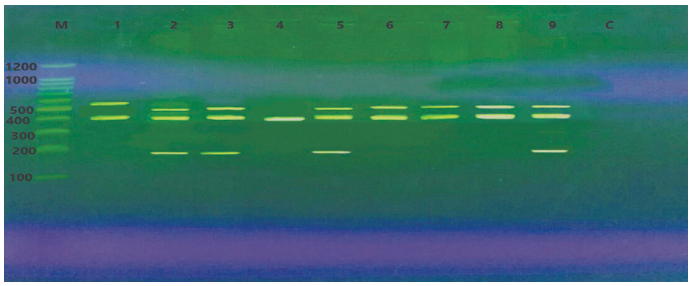

Figure 2: Results of electrophoresis of multiplex PCR for detection of *Campylobacter* spp. M: 100pb DNA marker, lane 1: *Campylobacter* isolate have cadF (400bp) and Iam1 (512bp), Lanes 2,3,5 and 9: *Campylobacter* isolates have cadF (400bp), cdtB (495bp) and cdtC (182bp), lane 4: *Campylobacter* isolates have cadF (400bp), lances 6, 7 and 8: Campylobacter isolates have cadF (400bp) and cdtB (495bp)

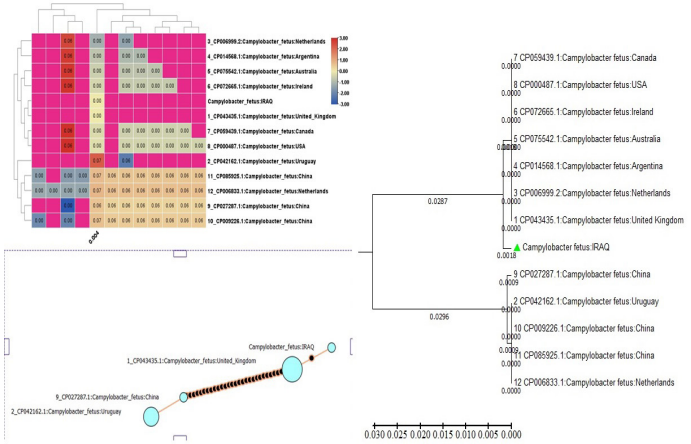

Figure 3: Phylogenetic tree of cadF gene from *Campylobacter* positive fetus.

The UPGMA method was used to infer evolutionary history, the optimum A tree having a total of branches lengths of 0.06369197 was depicted. The tree was displayed in scale, with lengths of branches (below the branches) in the same units as the evolutionary distances employed. To reconstruct the genomic tree, the evolutionary distances were calculated using the maximal composite likelihood approach (Tamura *et al*., 2013) and were expressed in terms of base substitutions made at each site. There were 13 nucleotide sequences in the analysis. The codon locations listed were first, second, third, non-coding. Every position with gaps or the missed information was deleted. The final dataset contained a total of 560 locations. MEGA6.0 was used to carry out evolutionary analyses (Igwaran and Okoh, 2020) Figure 3. The appearance of this virulence factors agreed with study conducted previously (Lluque *et al*., 2017; Ghunaim *et al*., 2015). The *cadF* is one of important genes, which is responsible for heat survival, and translate a protein necessary for adhesion and invasion (García-Sánchez *et al*., 2018). The *cdtB* genes is tripartite and fully functional toxin and plays function in causing harm to cells that caused dame in mucosa, Additionally, it has DNaseI-like activity which caused break down of double strand DNA (Al-Mutar, 2017). the *cdtC* gene is cytotoxin, causes cell distraction by DNA damage, chromatin and fragmentation (Ghorbanalizadgan *et al*., 2018). In the current study *C. jejuni* was isolated in low rate, proportionate to *Iam1* gene which was also detected in the lowest rate This supports the scientific references that refer to that Iam1 is frequency in *C. jejuni* (Rozynek *et al*., 2005).

CONCLUSION

Campylobacteriosis is the leading cause of ewe abortion in the area. *C. fetal* is the most pathogenic Campylobacter species in the studied region, affecting 17.5% of ewes.

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NOVELTY STATEMENT

This study introduces an innovative approach to clinical besides genetic analysis of bacteria in aborted ewes, and determines the antibiotic resistance.

AUTHOR'S CONTRIBUTION

This study was designed and written by the authors.

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None.

Data availability
The publication includes all datasets created and analyzed during the investigation.

Ethics statement
Not applicable.

Generative AI and AI-assisted technology statement
The authors acknowledge that the primary purpose of generative AI tools (such as ChatGPT and software language editing) is to improve formatting, grammar, and language clarity. No artificial intelligence (AI) techniques were employed for data interpretation, analysis, or scientific conclusion-making. The content of this article is entirely the writer's responsibility.

Conflict of interest
The author have declared no conflict of interest.

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