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RESEARCH ARTICLE

Pathological and Molecular Study of Campylobacter as Abortive Agent in Small Ruminants in Jordan

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A B S T R A C T

Abortion in small ruminants is one of the most prominent problems of livestock in many countries including Jordan. Our previous results regarding abortion revealed several undiagnosed cases with pathological lesions in placenta. This paper aims to expand our study to investigate the pathological lesions of *Campylobacter* as an abortive agent. Seventy-six aborted fetuses (17 goats and 59 sheep) and placenta of 53 fetuses were collected from farms in northern Jordan (2018 – 2019). The 76 available fetal liver and 53 placenta tissue were fixed with 10% NBF for histopathological and molecular investigations. Histopathological examination revealed that 17.11% cases had compatible histopathological lesions of *Campylobacter*. The 10.53% had multifocal hepatic necrosis with mild to moderate inflammatory reaction, 3 of which were positive using *Campylobacter* qPCR. On the other hand, qPCR detected 5 more positive cases but with no notable hepatic necrosis. All the placentas of the 8 qPCR positive cases had necrosuppurative placentitis. The age of 6 fetuses was estimated to be aborted at the third trimester and 2 at the second trimester based on the crown-rump length. This is the first pathological and molecular study to confirm the role of *Campylobacter* as abortive agent in small ruminants in Jordan.

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INTRODUCTION

Abortion in small ruminants is one of the most prominent problems of livestock in many countries including Jordan (Samadi *et al*., 2010). Diagnosis and control of abortions is very challenging specially in countries with insufficient capacities leading to heavy economic losses and social impacts (Brown *et al*., 2014; Bagheri Nejad *et al*., 2020). In addition, it is very clear that most abortions caused by infectious agents may also have zoonotic potential (Igwaran & Okoh, 2019). Previous studies have shown that Brucella and Chlamydia are the main known causes of abortions in sheep and goats in Jordan (Al-Qudah *et al*., 2004; Samadi *et al*., 2010). In another study, 40 fetuses and 25 placentas from aborted sheep and goat in Jordan were investigated to identify the causes of abortion using immunological, molecular and histopathological techniques; Brucellosis, Chlamydiosis were found. In the other hand, we found many undiagnosed cases, although they had pathological lesions, suggesting other causes of abortion (Hailat *et al*., 2017).

Campylobacter species are gram-negative microaerophilic bacteria that can be found in the intestine of many animals without showing any signs of disease. *Campylobacter* infection causes late term abortions and stillbirths in sheep and goats. Even if alive, newborns are weak and nonviable. *Campylobacter* species (*C. fetus subsp. fetus*, *C. jejuni subsp. jejuni* and *C. lari*) cause abortions in sheep more than goats (Njaa, 2012). Because Campylobacteriosis is highly contagious, most of the animals in the herd will be exposed to a highly organism-loaded placenta, fetus and abortion fluid (Wu *et al*., 2014). Up to the half of the pregnant naive ewes abort while the previously infected ewes are less likely to abort. The fetal and placental pathological findings were described naturally and experimentally, the main lesions were purulent placentitis with necrosis, bronchopneumonia and multifocal necrosis in liver (Njaa, 2012).

Campylobacteraceae family includes three genera: *Campylobacter*, *Arcobacter* and *Helicobacter*. The genus *Campylobacter* includes 22 species of gram negative, non-spore-forming microorganisms with a length between 0.5 to 5 μm and a width between 0.2 to 0.9 μm (Facciolà

et al., 2017). The major *Campylobacter* species that associated with sheep abortion outbreaks are *C. fetus subsp. fetus* and *C. jejuni subsp. jejuni*. Moreover, these two species associated, to a lesser extent, with sporadic abortions in goats and cattle (Skirrow, 1994; Moeller Jr, 2001; Sahin *et al*., 2012). The ingestion of food and water contaminated with faecal or aborted material (faecal-oral transmission) considered the natural route of infection for pregnant ewes (Skirrow, 1994). Clinical signs of Campylobacteriosis depend on at which stage of gestation the infection occurs and include abortion in the last trimester of gestation, stillbirths, and weak-born lambs. Aborting ewes may suffer from transient diarrhea and in a situation of the dead fetus is retained in the uterus, ewes may die due to septicemia (Skirrow, 1994; Njaa, 2012). The pale multifocal lesion of the fetal liver considered the most diagnostic lesion, but not pathognomonic, in aborted sheep. The most characteristic microscopic changes of the placenta are necrosuppurative placentitis, vasculitis and the presence of bacteria. The bacteria can be present in trophoblasts (intracytoplasmic and can be stained with Giemsa stain) and adjacent stroma and as large colonies within the remnants of vascular channels (Kirkbride, 1993; Sahin *et al*., 2008; Njaa, 2012; Sahin *et al*., 2017). Suppurative bronchopneumonia of the fetal lung is a common feature (Sahin *et al*., 2008; Njaa, 2012). The liver is characterized by multifocal lesions of coagulative necrosis surrounded by an infiltrate of mononuclear cells and neutrophils (Njaa, 2012). *Flexispira rappini* can also cause abortion in sheep and induced coagulative necrosis in the liver similar to that is found in *Campylobacter* infection. The gold standard for Campylobacter definitive diagnosis is the bacterial culture with microscopic findings described previously.

In humans who suffer from gastroenteritis, *Campylobacter jejuni* and *Campylobacter coli* are the most frequently *Campylobacter* species isolated. One of the important sources of human food-borne disease is the fecal contaminated poultry meat, where poultry is the main reservoir of this bacteria (Skarp *et al*., 2016; Rukambile *et al*., 2019; Alaboudi *et al*., 2020). The Jordanian Ministry of Health announced that between July 26th and 28th, 2020, more than 800 human food poisoning cases from Ain Al-Basha have been hospitalized. The causative agents of the food poisoning were *Enterococcus faecalis* and *Campylobacter* (Jordan Times, 2020). *Campylobacter* is a zoonotic bacterium that can cause animal and human disease (Hagos *et al*., 2019). In this paper, we report for the first time the pathological and molecular findings of Campylobateriosis in aborted fetuses from sheep and goats in Jordan.

MATERIALS AND METHODS

Sample collection: From different farms in northern governorates of Jordan, 76 aborted fetuses (17 goats and 59 sheep) were collected during the period between 2018 and 2019. Any clinical signs on the dam and complete history (farm and animal) were recorded. Complete gross examination was performed with emphasis on cotyledonary placenta and liver. Information about any gross pathological findings was recorded and all tissue samples, including the placenta and the fetal liver, lung, kidney, spleen, heart and brain, were fixed in 10% Naturally Buffered Formalin (NBF).

Histopathological Examination: Liver and placenta from aborted fetuses were fixed in 10% buffered formalin. Tissue samples were processed in the automatic tissue processor, embedded in paraffin wax, cut by trimming blade to a suitable size to be placed in a tissue cassette. Four to five µm thick sections of tissue samples were made by microtome. The sections were stained by Hematoxylin and Eosin (H&E) method (Suvarna *et al*., 2018) and were examined by light microscope.

Nucleic Acid Extraction: The DNA extraction from formalin-fixed paraffin-embedded (FFPE) tissue samples (53 samples from both liver and placenta and 23 samples from liver only) has been performed according to the Zymo-Research instruction manual (Quick-DNA FFPE Kit/ Catalog Nos. D3067). The eluted DNA was stored at-20°.

Real Time Polymerase Chain Reaction: Two qPCR assays were performed for the detection of *Campylobacter jejuni* and *Campylobacter fetus* in formalin-fixed paraffin-embedded (FFPE) fetal liver and/or placenta samples. The assays have been performed according to the manufacturer's instruction manuals (Genetic PCR Solutions). CamJej dtec-qPCR test and CamFet dtec-qPCR test for the detection of *Campylobacter jejuni* and *Campylobacter fetus*, respectively. The amplification regime steps are one cycle of activation (95°C), 40 cycles of denaturation (95°C) and one cycle of extension (60°C). The positive reaction was detected by the fluorescent signal accumulation and according to the number of cycles required to cross the threshold (Ct value), the sample that has Ct ≤35 was considered as positive while that has Ct >35 was considered as weak positive. Fig. 1 is showing the qPCR amplification curve of *C. jejuni* standards.

RESULTS

Table 1 shows that 17.11% cases had the histopathological lesions that are compatible with *Campylobacter*, 8 of which had multifocal hepatic necrosis and 10 had necrosuppurative placentitis (Fig. 3). Five cases had both multifocal hepatic necrosis and necrosuppurative placentitis. Analysis of the qPCR results revealed that 8 out of the 13 which had the pathological lesions were positive for Campylobacter. Furthermore, we found that 5 out of 76 (6.58%) cases were positive for *Campylobacter jejuni* and 3 out of 76 (3.95%) cases were positive for *Campylobacter fetus*. Five cases were positive (Ct value ≤ 35) while 3 cases were weak positive (Ct value > 35). Out of the five positive cases, 2 were *C. jejuni* and 3 were *C. fetus*. All the 3 weak positive cases were *C. jejuni*. The highest bacterial concentration (lowest Ct value = 28.24) was detected in the case that has grossly frank large multifocal hepatic necrosis (Fig. 2). Histopathological examination revealed that multifocal hepatic necrosis with mild to moderate inflammation was diagnosed in 8 out of 76 (10.53%) liver sections, 5 of which were small multifocal and 3 were

Table 1: Summary of the diagnostic results for the cases that had either positive qPCR result and/or multifocal hepatic necrosis.

Case Number	Histopathology		qPCR[3]		
	Multifocal Hepatic Necrosis[1]	Necrosuppurative Placentitis[2]	*C. jejuni*	*C. fetus*	Ct Value
12[4]	++	+	++	-	28.24
49	-	++	-	++	31.58
6	++	+	-	++	33.81
23	-	++	++	-	33.82
22	-	++	-	++	34.13
51	-	+	+	-	36.97
54	++	++	+	-	37.99
8	-	+	+	-	38.23
1	+	-	-	-	
18	+	-	-	-	
19	+	+	-	-	
20	+	+	-	-	
41	+	-	-	-	

1. Absent (-), small multifocal (+), large multifocal (++)
2. Absent (-), mild (+), severe (++)
3. Negative (-) weak positive (+), positive (++)
4. The only case that had large multifocal hepatic necrosis grossly (Fig. 2)

Hepatic necrosis and qPCR
qPCR only
Hepatic necrosis only

Table 2: The statistical results of the *Campylobacter* qPCR test

Species	Number of samples	Positive samples		Negative samples		*C. jejuni*	*C. fetus*
		No	(%)	No	(%)		
Sheep	59	7 (11.86%)		52 (88.14%)		4	3
Goat	17	1 (5.88%)		16 (94.12%)		1	0
Total	76	8 (10.53%)		68 (89.47%)		5	3

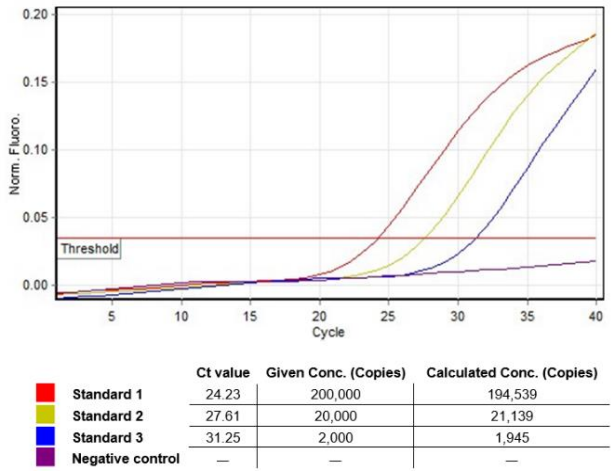

Fig. 1: qPCR amplification curve of *C. jejuni.*

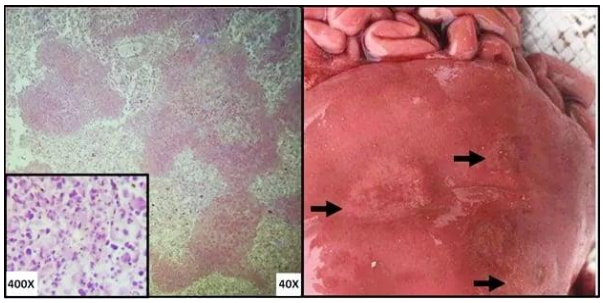

Fig. 2: Large multifocal to coalescing necrosis, liver, aborted fetus, sheep, H&E stain (Case 12).

Table 3: The gestational age estimates of the *campylobacter*-positive cases

Case #	Crown-Rump Length (cm)	Gestational Age (weeks)	Trimester	Species
6	11	7 to 9	Second	Sheep
8	48	19 to 21	Third	Goat
12	34	10 to 13	Second	Sheep
22	43	19 to 21	Third	Sheep
23	42	19 to 21	Third	Sheep
49	41	19 to 21	Third	Sheep
51	48	19 to 21	Third	Sheep
54	40	19 to 21	Third	Sheep

large. All the 3 cases that had large multifocal hepatic necrosis were positive on qPCR, 2 of which were *C. jejuni* and 1 was *C. fetus*. The 8 placentas of the qPCR positive cases had mild to severe necrosuppurative placentitis, while only 3 of which had vasculitis and 2 had bacterial colonies (Fig. 3, 4 & 5). Table 2 shows that 7 out of 59 (11.86 %) and 1 out of 17 (5.88 %) of sheep and goat's cases, respectively, were positive for *Campylobacter* using the qPCR test. The sheep positive cases were 4 *C. jejuni* and 3 *C. fetus* while the goat positive case was *C. jejuni*. Table 3 presents the gestational age estimates of the 8 *Campylobacter*-positive cases according to the crown-rump length (Njaa, 2012). Based on this data, we found that 6 out of the 8 positive cases (5 sheep and 1 goat) were aborted in the third trimester of gestation while 2 in the second trimester (1 sheep and 1 goat).

DISCUSSION

Analysis of the results revealed that *Campylobacter* is a cause of abortion in small ruminant in Jordan. It appears that the pathological lesions which were found in the fetal liver and placenta tissues are compatible with *Campylobacter*-abortion lesions that have been mentioned by Sahin *et al*. (2017) and Njaa (2012). However, these published reports indicate that *Campylobacter* can cause pneumonia, serositis, encephalitis and gastroenteritis in the aborted fetuses which we did not include in our study (Njaa, 2012; Sahin *et al*., 2017). As previously published regarding *campylobacter* abortion in small ruminants, it appears that *campylobacter* contributes significantly in the sheep abortion as account for 10.3-25.2% of all abortions (Sahin *et al*., 2017). Our results are in agreement with these published reports as 11.86% of aborted sheep cases were positive for *campylobacter* by qPCR. The most diagnostic gross lesion for *Campylobacter* is the pale multifocal necrotic areas of the fetal liver. This lesion is not pathognomonic, because when it is observed during abortion outbreak, *Campylobacter* is the likely cause. However, when it is observed in a sporadic case of

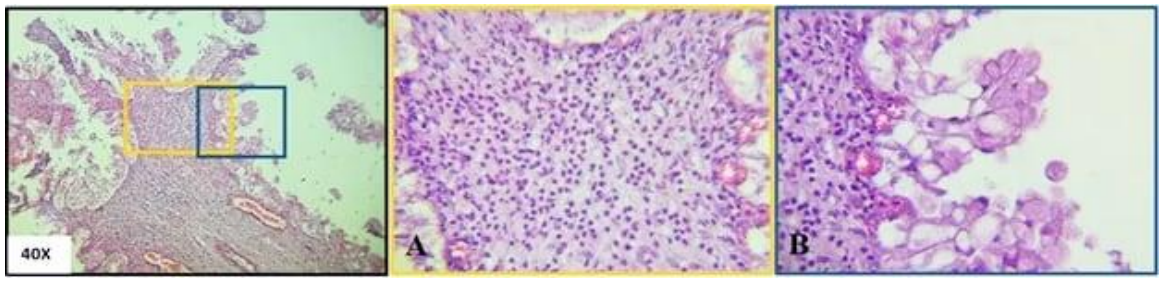

Fig. 3: Inflammation and necrosis of the chorionic villi, placenta, aborted fetus, sheep, H&E stain (Case 54): A, Lymphocyte-predominant inflammation of chorionic villi, 400X. B, Necrosis of trophoblasts, 400X.

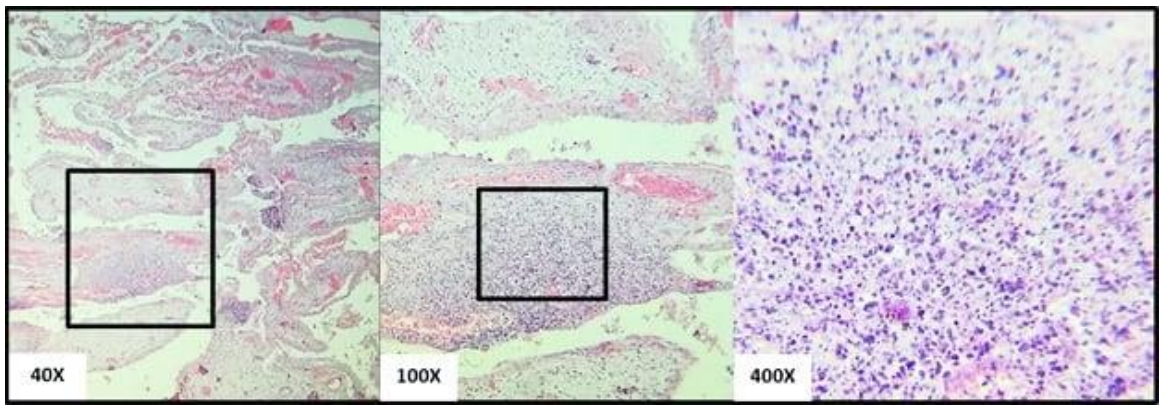

Fig. 4: Necrosis in the chorionic villi, placenta, aborted fetus, sheep, H&E stain (Case 49).

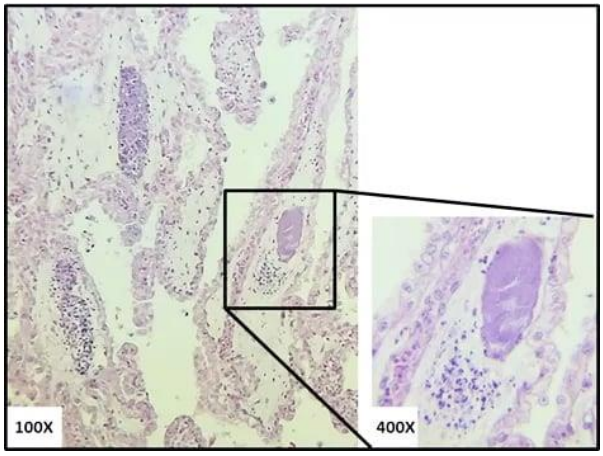

Fig: 5: Bacterial colony in the chorionic villi, placenta, aborted fetus, sheep, H&E stain (Case 23).

abortion, the likely cause is either *Campylobacter* or *Flexispira rappini*. Our results show that only 1 out of the 8 qPCR positive cases had this lesion during necropsy. This is compatible with the finding that the absence of this lesion does not rule out *Campylobacter* as the abortive cause (Kirkbride, 1993; Sahin *et al*., 2017). Previous studies showed that placentas are always affected in abortion cases caused by *Campylobacter* because it is the interconnection between the blood of the maternal circulation and the fetoplacental unit. The most characteristic histopathological changes of the placenta are necrosuppurative placentitis, vasculitis and the presence of bacterial colonies (Kirkbride, 1993; Sahin *et al*., 2008, 2017; Njaa, 2012). Our histopathological investigation of placental tissues showed that necrosuppurative placentitis, vasculitis and the presence of bacterial colonies were diagnosed in 8, 3 and 2 out of the 8 qPCR positive cases, respectively (Fig. 3, 4 & 5). With taking the different degrees of placental autolysis into consideration, these pathological changes could be masked. The higher percentage of *Campylobacter* qPCR positive cases in sheep (11.86%) than in goats (5.88%) corresponds with the finding that sheep are more susceptible to abortion by *Campylobacter* (Moeller Jr, 2001; Van Engelen *et al*., 2014). The gestational age estimation reveals that 6 out of the 8 (75%) qPCR positive cases were aborted at third trimester. This agrees with the finding that *Campylobacter* infections often causes a late-term abortion or stillbirth (Njaa, 2012). None of the qPCR positive cases were strong positive (relatively high Ct values ranged between 28.24 and 38.23). This might be attributed to the prolonged fixation (3-6 days) of the tissue samples in 10% NBF that might affect the DNA quality (Einaga *et al*., 2017). Four and three cases of *Campylobacter* abortion in sheep were positive for *Campylobacter jejuni* and *Campylobacter fetus* respectively. In this regard, *C. jejuni* is the most frequent species associated with ovine abortions in the United States compared with the United Kingdom, New Zealand and Australia where *Campylobacter fetus* is the most prevalent species (Fiorentino *et al*., 2017). In addition to the importance of *Campylobacter* prevention in avoiding economic losses, it is also important in public health (Johnson *et al*., 2017). In Jordan, a study conducted in 2018 revealed that 51 out of 368 (13.9%) human sera samples were positive for *C. jejuni* antibodies using ELISA. The risk factors analysis concluded that raw milk consumption and ruminant ownership are the most significant factors of human *Campylobacter* infection in Jordan (Obaidat, 2019). Since the prevention and control of abortion diseases are pathogen-specific, rapid and accurate etiological diagnosis is important. To our

knowledge, this work represents the first identification of *Campylobacter* as a cause of abortion in small ruminants in Jordan. Further works are needed in order to investigate *Campylobacter* as a cause of bovine abortion.

Conclusions and recommendations: This study represents the first molecular and pathological diagnosis of *Campylobacter* (*C. jejuni* and *C. fetus*) as a cause of abortion in sheep and goats in Jordan. The molecular results showed that 8 out of 76 (10.53%) of aborted sheep and goat fetuses were positive for *Campylobacter* using qPCR. The microscopic finding of multifocal hepatic necrosis in aborted sheep and goats' fetuses with the qPCR negative results for *C. jejuni* and *C. fetus* suggest further investigations on the presence of *C. lari* and *Flexispira rappini* as abortive agents of small ruminants in Jordan. Further work is needed in order to investigate *Campylobacter* as a cause of bovine abortion in Jordan.

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Authors contribution: NH: Results analysis, contribution in histology slides examination, contribution in proposal and manuscript writing. AA: Sample collection, contribution in histology slides preparation, contribution in histology slides examination, qPCR conducting, contribution in proposal and manuscript writing. MA: qPCR supervision, qPCR results analysis.

REFERENCES

Alaboudi AR, Malkawi IM, Osaili TM, *et al*., 2020. Prevalence, antibiotic resistance and genotypes of *Campylobacter jejuni* and *Campylobacter coli* isolated from chickens in Irbid governorate, Jordan. Int J Food Microbiol 11:108656.

Al-Qudah KM, Sharif LA, Raouf RY, *et al*., 2004. Seroprevalence of antibodies to Chlamydophila abortus shown in Awassi sheep and local goats in Jordan. Veterinarni Medicina-UZPI (Czech Republic).

Bagheri Nejad R, Krecek RC, Khalaf OH, *et al*., 2020. Brucellosis in the Middle East: Current situation and a pathway forward. PLOS Neg Trop Dis 14:e0008071.

Brown C, Garin-Bastuji B, Ziay G, *et al*., 2014. Improving the diagnostic capacities of Brucellosis, enhancing the vaccination and control strategies in the Middle East and North Africa. Pak Vet J 34:263-4.

Einaga N, Yoshida A, Hiroko Noda H, *et al*., 2017. Assessment of the quality of DNA from various formalin-fixed paraffin-embedded (FFPE) tissues and the use of this DNA for next-generation sequencing (NGS) with no artifactual mutation. PLoS One 12:1-18.

Facciolà A, Riso R, Avventuroso E, *et al*., 2017. Campylobacter: from microbiology to prevention. J Prev Med Hyg 58:E79.

Fiorentino MA, Stazionati M, Hecker Y, *et al*., 2017. Campylobacter fetus subsp. fetus ovine abortion outbreak in Argentina. Rev Elect de Vet 18:1-1.

Hagos Y, Berhe M and Gugsa G, 2019. Campylobacteriosis: Emphasis on its Status as Foodborne Zoonosis in Ethiopia. J Trop Dis 7:2.

Hailat N, Khlouf S, Ababneh M, *et al*., 2017. Pathological, Immunohistochemical and Molecular Diagnosis of Abortions in Small Ruminants in Jordan with Reference to Chlamydia abortus and Brucella melitensis. Pak Vet J 38:109-12.

Igwaran A and Okoh AI, 2019. Human campylobacteriosis: a public health concern of global importance. Heliyon 5:e02814.

Johnson TJ, Shank JM and Johnson JG, 2017. Current and potential treatments for reducing Campylobacter colonization in animal hosts and disease in humans. Front Microbiol 8:487.

Jordan Times, 2020. Five-Year-Old Boy Dies, Over 800 Hospitalised After Food Poisoning. [online] Available at: http://jordantimes.com/news/local/five-year-old-boy-dies-over-800-hospitalised-after-food-poisoning> [Accessed 7 September 2020].

Kirkbride CA, 1993. Diagnoses in 1,784 ovine abortions and stillbirths. J Vet Diagn Invest 5:398-402.

Moeller Jr RB, 2001. Causes of caprine abortion: diagnostic assessment of 211 cases (1991–1998). J Vet Diagn Invest 13:265-70.

Njaa BL, 2012. Kirkbride's diagnosis of abortion and neonatal loss in animals. John Wiley & Sons.

Obaidat MM, 2019. Seroprevalence and risk factors for Campylobacter jejuni seropositivity in Jordan. Infect Dis 51:140-6.

Rukambile E, Sintchenko V, Muscatello G, *et al*., 2019. Infection, colonization and shedding of Campylobacter and Salmonella in animals and their contribution to human disease: A review. Zoon Pub Health 66:562-78.

Sahin O, Fitzgerald C, Stroika S, *et al*., 2012. Molecular evidence for zoonotic transmission of an emergent, highly pathogenic Campylobacter jejuni clone in the United States. J Clin Microbiol 50:680-7.

Sahin O, Plummer PJ, Jordan DM, *et al*., 2008. Emergence of a tetracycline-resistant Campylobacter jejuni clone associated with outbreaks of ovine abortion in the United States. J Clin Microbiol 46:1663-71.

Sahin O, Yaeger M, Wu Z,*et al*., 2017. Campylobacter-associated diseases in animals. Annual Rev Animal Biosci 5:21-42.

Samadi A, Ababneh M, Giadinis ND, *et al*., 2010. Ovine and caprine brucellosis (Brucella melitensis) in aborted animals in Jordanian sheep and goat flocks. Vet Med Int 2010: 458695

Skarp CP, Hänninen ML and Rautelin HI, 2016. Campylobacteriosis: the role of poultry meat. Clin Microbiol Infect 22:103-9.

Skirrow MB, 1994. Diseases due to Campylobacter, Helicobacter and related bacteria. J Comp Pathol 111:113-49.

Suvarna KS and Layton C, 2018. Bancroft's Theory and Practice of Histological Techniques E-Book: Elsevier Health Sciences.

Van Engelen E, Luttikholt S, Peperkamp K, *et al*., 2014. Small ruminant abortions in The Netherlands during lambing season 2012–2013. Vet Rec 174:506.

Wu Z, Sippy R, Sahin O, *et al*., 2014. Genetic diversity and antimicrobial susceptibility of Campylobacter jejuni isolates associated with sheep abortion in the United States and Great Britain. J Clin Microbiol 52:1853-61.