Original Article

Identification and genotyping of *Chlamydia abortus* with MLVA from ruminant abortions in the Marmara region of Turkey

Mehmet Engin MALAL[1*] Suheyla TURKYILMAZ[2]

Abstract

Abortion in ruminants is an important problem in Turkey and it leads to serious economic losses for farmers. The aim of this study is to diagnose *Chlamydia abortus* in ruminant abortions in the Marmara Region of Turkey and to determine the genotypes of the agent and obtain the first national epidemiological data in this sense. For this purpose, a total of 730 abortion materials (fetal tissue, fetal stomach contents, placentas, cotyledons, vaginal swabs) belonging to 267 cattle, 380 sheep, 70 goats and 13 water buffaloes were examined for *C. abortus* with species specific real-time PCR. DNAs of positive samples were genotyped by the Multilocus Variable Number Tandem Repeat Analysis (MLVA) method. From 730 materials, 87 (11.9%) were found positive for *C. abortus*. Positivity rates were 21.4% in goats, 16.6% in sheep, 7.7% in water buffaloes and 3% in cattle. The dominant genotype was found to be MLVA genotype 2 (93.1%), and 4 different genotypes including genotypes 3, 4, and 5 were involved in infections. So it is concluded that genotypic diversity of *C. abortus* is high in the Marmara region. It was also revealed that *C. abortus* was responsible for a significant proportion of small ruminant abortions in this region.

Keywords: *Chlamydia abortus*, Genotyping, MLVA, Real Time PCR, VNTR, Turkey

[1]*Pendik Veterinary Control Institute, Istanbul, Turkey*
[2]*Adnan Menderes University, Veterinary Faculty, Microbiology Department, Aydın, Turkey*
***Correspondence**: engin_malal@hotmail.com* (M. E. MALAL)
Received October 23, 2020.
Accepted January 8, 2021.
doi: 10.14456/tjvm.2021.22

Introduction

Chlamydia abortus causes an abortigenic disease called "chlamydiosis" or "enzootic abortion" in ruminants, especially in sheep and goats (Szeredi and Bacsadi 2002; Givens and Morally 2008). The disease is characterized by abortion in the last 2-3 weeks of pregnancy, premature birth, low birth weight and neonatal death within 48 hours after birth (Givens and Morally 2008).

The disease repeats periodically in the herd and is very difficult to control. When factors such as loss of offspring due to the disease, treatment expenses and decrease in milk production are taken into account, the disease causes significant economic loss (Longbottom *et al*., 2002, Carter and Wise 2004). The disease also poses a risk to public health (Ward 2006). The causative agent is the most important cause of sheep and goat abortions in many regions of the world and shows an endemic course (Essig and Longbottom 2015).

The prevalence of the disease is similar in Turkey's neighbors as well. In molecular studies, it has been revealed that very high proportions (35.8%) of small ruminant abortions in Bulgaria are caused by *C. abortus* (Simeonov and Chilingirova 2018). In Iran, *C. abortus* has been detected in 11.0% to 38.0% of the small ruminant abortions (Ebadi *et al*., 2015, Barati *et al*., 2017, Heidari *et al*., 2017). In serological studies conducted in Greece, 14.9% and 21.2% seropositivity has been detected in sheeps and goats (Bisias *et al*., 2010) and 2 variant strains (LLG, POS), which are genotypically different from those of the whole world, have been identified (Siarkau *et al*., 2002, Laroucau *et al*., 2009).

C. abortus is genetically very homogeneous. The most preferred methods for genotyping *C. abortus* are Multilocus Sequence Typing (MLST) and Multilocus Variable Number Tandem Repeat Analysis (MLVA). When these methods are compared and contrasted, while MLST method can classify C. *abortus* into 5 different genotypes, 7 different genotypes can be obtained with MLVA (Siarkou *et al*., 2015). In addition, the MLVA method is suitable for direct typing from abortion material (Laroucau *et al*., 2009).

In Turkey, general serological studies have been carried out for this disease. Relatively high seropositivity rates have been seen in sheep such as 20.8% in Duzce (Karagül *et al*., 2019) and 32.0% (Öztürk *et al*., 2016) in Burdur. These results can be attributed to the agent as there are no vaccinations carried out in Turkey. Although the existence of the agent has been revealed by molecular studies and isolation studies, these studies far from present sufficient data on the prevalence of the disease in the national sense. The first study on the isolation of *C. abortus* in Turkey was carried out by Turutoglu and Erganis (1996) and it was reported that 16.3% isolation was obtained from sheep abortion materials. Guler *et al*., (2006) and Kalender *et al*., (2013) found 7.4% and 9.8% positivity from sheep abortions with the help of conventional PCR. There is no data regarding *C. abortus* genotypes in Turkey. However, it may be thought that the genotypic diversity was high due to its geographical location. The aim of this study is to diagnose *C. abortus* from ruminant abortions in the Marmara region of Turkey by real-time PCR and genotype with MLVA.

Materials and Methods

Ethical considerations: Ethical approval for the study was obtained from the Local Ethics Committee for Animal Experiments, Pendik Veterinary Control Institute (RPN 07/2018), Istanbul, Turkey.

Samples: In the study, samples from ruminant abortion cases obtained between 2017-2019 in the provinces in the working area of Istanbul Pendik Veterinary Control Institute (Bilecik, Istanbul, Edirne, Canakkale, Tekirdag, Kirklareli, Bursa, Balikesir, Duzce, Yalova, Kocaeli, Sakarya) (Figure 1) were used.

The material of the study consisted of fetal stomach contents and internal organs of the fetus (lungs, liver, kidneys and spleen), placental tissue and vaginal swabs. For this purpose, 267 cattle, 380 sheep, 70 goats and 13 water buffalo samples belonging to 730 abortion cases were analyzed

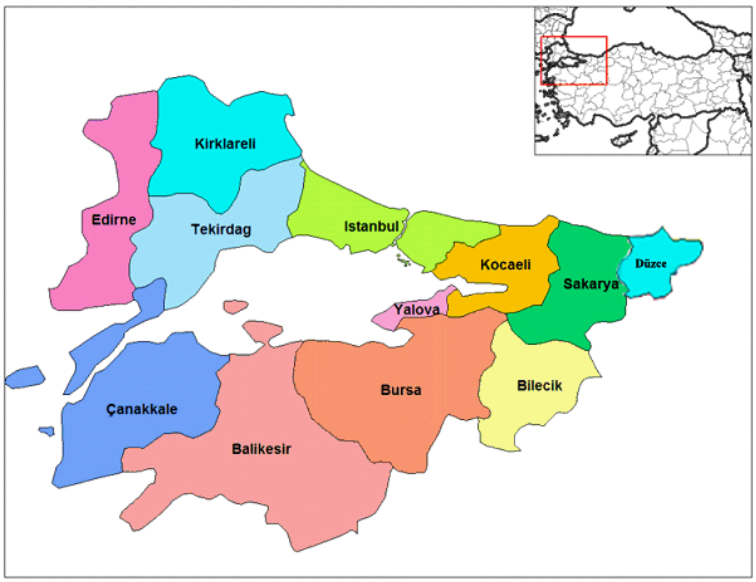

Figure 1 Provinces where samples were taken.

DNA extraction and real-time PCR: DNA extraction was performed with DNeasy® Blood & Tissue Kit (Qiagen), according to the manufacturer's directions. Fetal tissues and stomach contents, placental tissue and vaginal swabs were used for DNA extraction. Real-time PCR analysis was performed as previously described (Pantchev *et al*., 2009). Selected primers which are specific to *C. abortus* DNA were utilized for the amplification of the *omp*A gene region. Each reaction mix contained 0.9 µM of each primer, 0.2 µM of probe and 2 µl of template DNA. The amplification cycle included 15-minute denaturation at 95^{0}C followed by 45 cycles at 95^{0}C for 15s and 60^{0}C for 60s. DNA of *C. abortus* S26/3 reference strain was used as the positive control and nuclease-free water as the negative control.

MLVA: MLVA was performed with template DNAs of the samples found positive with real time PCR. For MLVA, Laroucau *et al*., (2009)'s method had been modified. The reaction was carried out with 10 µl 2x mastermix (Norgen, Canada), 1 µl 10 qmol primers, 5 µl nuclease-free water and 3 µl template DNA in a total of 20 µl mix. The temperature time profile was 95^{0}C 15 m of denaturation followed by 40 cycles at 95^{0}C 30s, at 58^{0}C 30s, at 72^{0}C 45s, and final extention at 72^{0}C 10 m. Five VNTR loci, (ChlAb 300, ChlAb 457, ChlAb 581, ChlAb 620, and ChlAb 914) were used as the genotyping markers. Primers described previously for MLVA were used. However, the 5 ' ends of the forward primers were marked with Fam-6. After PCR, products were stored at -20^{0}C until capillary gel electrophoresis. Capillary gel electrophoresis was performed to obtain amplicon sizes at Pendik Veterinary Control Institute using ABI 3130 XL (Applied Biosystems). The number of tandem repeats of 5 gene regions was found and MLVA genotypes were identified (Laroucau *et al*., 2009).

Statistical Analysis: The Pearson Chi-square test was run for both small ruminants and all the samples to identify whether there was a significant difference between provinces of the Marmara Region, Turkey. For the analysis, SPSS 18 (Statistical Package for Social Sciences) was utilized.

Results

Real Time PCR: *C. abortus* DNA was detected by real-time PCR from 87 (11.9%) of the 730 materials. 15 of 70 (21.4%) goat samples, 63 of 380 (16.6%) sheep samples, 1 of 13 (7.7%) water buffalo samples, 8 of 267 (3.0%) cattle samples were found to be positive. Real Time PCR results according to province and animal species are given in Table 1.

Table 1 Real-time PCR results

Province	Sheep	Positive	Goats	Positive	Cattle	Positive	Water Buffalo	Positive	Total Sample	Total Positive (%)
Balikesir	37	6	2	-	32	1	-	-	**71**	**7 (9.8)**
Bilecik	9	-	3	1	5	-	-	-	**17**	**1 (5.9)**
Bursa	58	7	11	1	26	1	2	-	**97**	**9 (9.3)**
Canakkale	81	16	21	2	41	1	-	-	**143**	**19 (13.3)**
Duzce	3	1	-	-	32	1	4	1	**39**	**3 (7.7)**
Edirne	85	16	6	3	66	2	-	-	**157**	**21 (13.4)**
Istanbul	7	3	5	1	3	-	-	-	**15**	**4 (26.7)**
Kirklareli	49	11	14	5	17	-	-	-	**80**	**16 (20)**
Kocaeli	14	1	5	2	11	-	7	-	**37**	**3 (8.1)**
Sakarya	7	-	1	-	10	-	-	-	**18**	**0 (0)**
Tekirdag	25	2	2	-	19	1	-	-	**46**	**3 (6.5)**
Yalova	5	-	-	-	5	1	-	-	**10**	**1 (10)**
Total	**380**	**63 (16.6%)**	**70**	**15 (21.4%)**	**267**	**8 (3%)**	**13**	**1 (7.7%)**	**730**	**87(11.9)**

The results of the Pearson Chi-square test indicated that there was no significant difference between cities and small ruminants and also between cities and all of the animals (Table 2).

Genotyping: Genotypes of all 87 *C. abortus* positive samples were determined by MLVA (Figure 2). 81 out of 87 (93.1%) samples were found to be genotype 2. Genotype 2 was followed by genotype 5 with 4 (4.6%) samples, genotype 4 with 1 (1.1%) sample and genotype 3 with 1 (1.1%) sample. Genotypic classification according to animal species is given in Table 3.

Table 2 Statistical evaluation of positive results.

	χ2 value	P value
Provinces and Positivity (Small Ruminants)	13.326	0.273
Provinces and Total Positivity	15.540	0.159

No	Sample ID	Host	Province	Town	ChlaAb-300	ChlaAb-457	ChlaAb-581	ChlaAb-620	ChlaAb-914	Genotyping result
1	2017-75-1	Goat	Istanbul	Kadikoy	3	1	1	2	1	MLVA genotype 2
2	2017-579-1	Sheep	Canakkale	Can	3	1	1	2	1	MLVA genotype 2
3	2017-929-1	Goat	Kirklareli		3	1	1	2	1	MLVA genotype 2
4	2017-1421-1	Goat	Canakkale	Ayvacik	3	1	1	2	1	MLVA genotype 2
5	2017-1590-1	Cattle	Tekirdag	Hayrabolu	3	1	1	2	1	MLVA genotype 2
6	2017-1625-1	Cattle	Yalova		3	1	2	3	1	MLVA genotype 4
7	2017-1726-1	Sheep	Kirklareli		3	1	1	2	1	MLVA genotype 2
8	2017-1728-1	Sheep	Edirne	Uzunkopru	3	1	1	2	1	MLVA genotype 2
9	2017-2289-1	Sheep	Edirne	Lalapasa	3	1	1	2	1	MLVA genotype 2
10	2017-2420-1	Goat	Edirne	Uzunkopru	3	1	1	2	1	MLVA genotype 2
11	2017-2522-1	Sheep	Canakkale	Can	3	1	1	2	1	MLVA genotype 2
12	2017-2685-1	Sheep	Tekirdag	Malkara	3	1	1	2	1	MLVA genotype 2
13	2017-4082-1	Sheep	Canakkale	Yenice	3	1	1	2	1	MLVA genotype 2
14	2017-4913-1	Sheep	Bursa	Karacabey	3	1	1	2	1	MLVA genotype 2
15	2017-5203-1	Sheep	Canakkale	Gelibolu	3	1	1	2	1	MLVA genotype 2
16	2017-6123-1	Cattle	Edirne		3	1	1	2	1	MLVA genotype 2
17	2017-6198-1	Cattle	Bursa		3	1	1	2	1	MLVA genotype 2
18	2017-8228-1	Sheep	Edirne		3	1	1	2	1	MLVA genotype 2
19	2017-7693-1	Sheep	Edirne		3	1	1	2	1	MLVA genotype 2
20	2017-7762-1	Sheep	Kirklareli		3	1	1	2	1	MLVA genotype 2
21	2017-9148-1	Sheep	Canakkale	Ezine	3	1	1	2	1	MLVA genotype 2
22	2017-9431-1	Sheep	Canakkale	Ezine	3	1	2	3	2	MLVA genotype 5
23	2017-9866	Sheep	Bursa		3	1	1	2	1	MLVA genotype 2
24	2017-10164	Sheep	Bursa		3	1	1	2	1	MLVA genotype 2
25	2017-10590	Goat	Kirklareli		3	1	1	2	1	MLVA genotype 2
26	2017-10774	Sheep	Canakkale	Yenice	3	1	1	2	1	MLVA genotype 2
27	2017-10880	Sheep	Balikesir	Kepsut	3	1	1	2	1	MLVA genotype 2
28	2017-10833	Sheep	Canakkale	Ezine	3	1	1	2	1	MLVA genotype 2
29	2017-10945	Sheep	Istanbul	Catalca	3	1	1	2	1	MLVA genotype 2
30	2017-11118	Sheep	Edirne		3	1	1	2	1	MLVA genotype 2
31	2017-11119	Sheep	Canakkale	Yenice	3	1	1	2	1	MLVA genotype 2
32	2017-11256	Sheep	Edirne	Ipsala	3	1	1	2	1	MLVA genotype 2
33	2017-11350	Sheep	Kirklareli		3	1	1	2	1	MLVA genotype 2
34	2017-11427	Sheep	Bursa	Mustafakemalpasa	3	1	1	2	1	MLVA genotype 2
35	2018-1-1	Sheep	Duzce	Golyaka	3	1	1	2	1	MLVA genotype 2
36	2018-164-1	Sheep	Kirklareli	---	3	1	1	2	1	MLVA genotype 2
37	2018-331-1	Sheep	Kirklareli	---	3	1	1	2	1	MLVA genotype 2
38	2018-332-1	Sheep	Edirne	---	3	1	1	2	1	MLVA genotype 2
39	2018-338-1	Goat	Edirne	Lalapasa	3	1	1	2	1	MLVA genotype 2
40	2018-339-1	Sheep	Canakkale	---	3	1	1	2	1	MLVA genotype 2
41	2018-462-1	Sheep	Kirklareli	---	3	1	1	2	1	MLVA genotype 2
42	2018-478-1	Cattle	Duzce	---	3	1	1	2	1	MLVA genotype 2
43	2018-501-1	Sheep	Balikesir	---	3	1	1	2	1	MLVA genotype 2
44	2018-524-1	Sheep	Istanbul	Sile	3	1	1	2	1	MLVA genotype 2
45	2018-587-1	Sheep	Istanbul	Catalca	3	1	1	2	1	MLVA genotype 2
46	2018-616-1	Goat	Kirklareli	---	3	1	1	2	1	MLVA genotype 2
47	2018-738-1	Sheep	Edirne	Havsa	3	1	1	2	1	MLVA genotype 2
48	2018-813-1	Goat	Kirklareli	---	3	1	1	2	1	MLVA genotype 2
49	2018-813-2	Goat	Kirklareli	---	3	1	1	2	1	MLVA genotype 2
50	2018-880-1	Goat	Canakkale	Ezine	3	1	1	2	1	MLVA genotype 2
51	2018-940-1	Sheep	Edirne	---	3	1	1	2	1	MLVA genotype 2
52	2018-1069-1	Sheep	Canakkale	Biga	3	1	1	2	1	MLVA genotype 2
53	2018-1113-1	Sheep	Edirne	Ipsala	3	1	1	2	1	MLVA genotype 2
54	2018-1306-1	Sheep	Bursa	---	3	1	1	2	1	MLVA genotype 2
55	2018-1351-1	Goat	Kocaeli	Karamursel	3	1	1	2	1	MLVA genotype 2
56	2018-1444-1	Sheep	Canakkale	Gelibolu	3	1	1	2	1	MLVA genotype 2
57	2018-1552-1	Water buffalo	Duzce	Merkez	3	1	2	2	1	MLVA genotype 3
58	2018-1560-1	Sheep	Bursa	---	3	1	1	2	1	MLVA genotype 2
59	2018-1741-1	Goat	Bilecik	Sogut	3	1	1	2	1	MLVA genotype 2
60	2018-1832-1	Sheep	Balikesir	Kepsut	3	1	1	2	1	MLVA genotype 2
61	2018-1844-1	Sheep	Edirne	Lalapasa	3	1	1	3	2	MLVA genotype 5
62	2018-1911-1	Sheep	Canakkale	Gelibolu	3	1	1	2	1	MLVA genotype 2
63	2018-10863-1	Sheep	Kirklareli		3	1	1	2	1	MLVA genotype 2
64	2018-2038-1	Cattle	Canakkale	Biga	3	1	1	2	1	MLVA genotype 2
65	2018-2612-1	Sheep	Canakkale	Gelibolu	3	1	1	2	1	MLVA genotype 2
66	2018-2576-1	Sheep	Balikesir	Bandirma	3	1	1	2	1	MLVA genotype 2
67	2018-3142-1	Cattle	Edirne	Merkez	3	1	1	2	1	MLVA genotype 2
68	2018-4749-1	Sheep	Kirklareli	---	3	1	1	2	1	MLVA genotype 2
69	2018-7803-1	Sheep	Canakkale	Ezine	3	1	1	2	1	MLVA genotype 2
70	2018-7896-1	Sheep	Edirne	Lalapasa	3	1	1	2	1	MLVA genotype 2
71	2018-8203-2	Sheep	Tekirdag	Marmaraereglisi	3	1	1	2	1	MLVA genotype 2
72	2018-8692-1	Sheep	Edirne	---	3	1	1	2	1	MLVA genotype 2
73	2018-8722-1	Sheep	Edirne	---	3	1	1	2	1	MLVA genotype 2
74	2018-8888-1	Sheep	Balikesir	Altieylul	3	1	1	2	1	MLVA genotype 2
75	2018-8902-1	Sheep	Edirne	---	3	1	1	2	1	MLVA genotype 2
76	2018-9527-1	Sheep	Balikesir	Altieylul	3	1	1	2	1	MLVA genotype 2
77	2018-10150-1	Sheep	Kirklareli	---	3	1	1	2	1	MLVA genotype 2
78	2018-10257-1	Sheep	Kirklareli	---	3	1	2	3	2	MLVA genotype 5
79	2018-10439-1	Sheep	Bursa	---	3	1	1	2	1	MLVA genotype 2
80	2019-36-1	Goat	Bursa	---	3	1	1	2	1	MLVA genotype 2
81	2019-263-1	Goat	Edirne	Ipsala	3	1	1	2	1	MLVA genotype 2
82	2019-783-1	Goat	Kocaeli	---	3	1	1	2	1	MLVA genotype 2
83	2019-826-1	Sheep	Kocaeli	Gebze	3	1	1	2	1	MLVA genotype 2
84	2019-857-1	Sheep	Edirne	Lalapasa	3	1	1	2	1	MLVA genotype 2
85	2019-1444-1	Sheep	Canakkale	Ezine	3	1	1	2	1	MLVA genotype 2
86	2019-2518-1	Sheep	Kirklareli	---	3	1	1	2	1	MLVA genotype 2
87	2019-3705-1	Cattle	Balikesir	---	3	1	2	3	2	MLVA genotype 5
	Reference Strain S26/3				3	1	2	3	2	MLVA genotype 5

Figure 2 MLVA genotyping results

Table 3 Genotypic classification according to animal species.

Species	Genotype 2	Genotype 3	Genotype 4	Genotype 5
Sheep	60 (%95.2)			3 (%4.8)
Goats	15 (%100)			
Cattle	6 (%75)		1 (%12.5)	1 (12.5)
Water Buffalo		1 (%100)		

Discussion

Abortions caused by infectious agents in ruminants cause significant economic loss. Although the leading abortigenic agent in Turkey is *Brucella* spp., serological studies have shown that *C. abortus* may also be of high importance (Ozturk *et al*., 2016; Karagul *et al*., 2019). Different methods are used in the diagnosis of the disease but detection of *Chlamydiaceae* can be carried out more specifically by DNA-based methods (Longbottom *et al*., 2001). One of the preferred methods to get the most reliable and rapid results is real-time PCR (Sachse *et al*., 2009). In this study, the real-time PCR method, which can identify *C. abortus* as species-specific and which has a low detection limit, was preferred (Pantchev *et al*., 2009). Eighty-seven (11.9%) positive results were obtained from a total of 730 samples. The highest positivity rate was found in goats with 21.4% and in sheep with 16.6%. When the previous studies, including small ruminants in Turkey are examined, Turutoglu and Erganis (1996) isolated *C. abortus* from 16.3% of the sheep abortion materials. This finding is in line with our study. There have been some studies which also used the conventional PCR method. Guler *et al*., (2006) detected *C.abortus* DNA in 7.5% of the vaginal swabs taken from 15 sheep flocks. Kalender *et al*., (2013) detected 9.8% chlamydial DNA in 64 sheep abortions in North Anatolian Turkey. The positivity rates in sheep in our study are higher than those of the two studies. This difference might stem from the sampling way, analysis method used and the regions.

When the studies carried out in contexts other than Turkey are taken into account, in a study conducted in Switzerland, 39% of sheep abortions and 23% of goat abortions were found to have originated from *C. abortus* (Chanton-Greutmann *et al*., 2002). While the percentage belonging to goats in our study, is very close to their result (21.4%). The percentage was lower in sheep (16.3%). In the Iranian context, one of Turkey's neighbours, different positivity rates (11-37%) were obtained from sheep and goats by PCR (Ebadi *et al*., 2015; Barati *et al*.,2017; Heidari *et al*., 2017). It can be said that in these contexts positivity rates are generally higher than the ones in our study.Abortion cases in sheep and goats between 2013 and 2018 in Bulgaria, another neighbour of Turkey, were investigated with PCR for *C. abortus* and 35.8% positivity was found. The positivity rates in sheep and goats were 41.9% and 25%, respectively (Simeonov and Chilingirova, 2018). In Bulgaria, the results belonging to goats are close to the ones in our study; however, the positivity rates of sheep are a lot higher.

In our study, cattle and water buffalo samples were also investigated. The positivity rate was 3% in cattle while *C. abortus* DNA was detected in a single water buffalo sample. In a study, Temur *et al*., (2007) determined the agent (5.5%) with the modified Ziehl-Neelsen staining method following egg inoculation from cattle abortions in Erzurum, Turkey. Kılıç *et al*., (2010) determined the DNA of the agent in 6.3% of the bovine abortions. Aras *et al*., (2017) determined 3% *C. abortus* positivity by the PCR method from bovine abortion cases in Konya and Aksaray, Turkey. Positivity rates in bovine abortions in our study showed similarity with these studies. Similar to the results of our study, in cattle abortion cases in Switzerland between 2003-2004, *C. abortus* was detected molecularly in 12 of 235 samples (5.1%) (Borel *et al*., 2006). Osman *et al*., (2012) investigated the presence of *Chlamydiaceae* in cattle in Egypt and identified *C. abortus* DNA in 15.1% of 73 cattle samples. This rate was higher than the cattle positivity in our study (3.0%).

Although *Chlamydia* species are known to infect water buffaloes (Burnard and Polkinghorne, 2016; Galiero, 2007), studies using molecular detection are limited. Greco *et al*., (2008) diagnosed *Chlamydia* spp. by nesting PCR in a flock with an abortion rate of 36.8% and detected in 21.4% of 14 vaginal swab samples and 42.9% of 7 fetuses. It was revealed that two of the fetuses were simultaneously infected with *C. abortus* and *Chlamydia pecorum* and one was infected with *C. abortus* only. In Egypt, 9.8% of 102 water buffalo samples were found to be positive (Osman *et al*., 2012). The low number of water buffalo samples (13) in our study makes it impossible to compare the results with other studies. However, this study is important in terms of the fact that *C. abortus* was detected in a water buffalo fetus for the first time in Turkey.

When sheep and goats, which are the most affected species, were evaluated together, *C. abortus* DNA was detected in 78 of 450 materials (17.3%). This rate falls to 3.2% in cattle and water buffaloes together. These rates show that the disease is more important in small ruminants both in the Marmara Region and the world (Longbottom and Coulter, 2003). *C. abortus* was detected in Istanbul, Edirne, Kirklareli, Tekirdag, Canakkale, Kocaeli, Yalova, Bilecik, Bursa and Balikesir provinces. The only province in which there was no positive result was Sakarya. However, it was evaluated that the negative result could be misleading because of the number of materials provided (18). Additionally, the majority of them (10) were cattle samples with low positivity rates. The difference between positivity rates and provinces was not statistically significant, which shows that there is not a

single province which is affected by the disease more severely than the others; in fact, the whole region is affected by the disease.

It was observed that 81 of 87 (93,1%) *C. abortus* DNA genotyped in the Marmara Region was genotype 2. From 199 vaginal swabs belonging to sheep and goats in Algeria, 6.5% *C. abortus* positivity was found and all of them genotyped as genotype 2 with MLVA (Merdja *et al*., 2015). Yak abortion cases in China were investigated for *C. abortus*; 23.8% positivity was found in 9 abortion fetuses and 126 vaginal swabs and all of them were found to be MLVA genotype 2 (Li *et al*., 2015). In France, 88.9% of 9 *C. abortion* strains isolated from clinical samples were found to be genotype 2. In contrast, only genotype 4 and genotype 5 have been identified in the UK so far (Laroucau *et al*., 2009). These studies generally show that the dominant MLVA genotype in the world is genotype 2 and these results are also compatible with our study. However, in our study, it was observed that genotypes 3, 4 and 5 also play roles in infections in the Marmara Region, so it can be concluded that *C. abortus* has high genotypic diversity in Turkey.

It was observed that the dominant genotype is genotype 2 in cattle, sheep and goats. 95.2% of 63 sheep, all 16 goats and 75% of 8 cattle samples were classified as genotype 2. The only positive water buffalo sample was found to be genotype 3. Since there is only one positive sample, an assessment of the common genotype in water buffalo infections is not possible. However, it is remarkable that a single water buffalo isolate was found to be genotype 3, which is not a common genotype and no genotype 3 was detected in any other animal species. Due to the fact that we didn't encounter any other studies of genotyping of water buffalo isolate with MLVA, we could not make a comparison. One cattle sample was found to be genotype 4 and one to be genotype 5. While genotype 4 was not detected from sheep and goat samples, 3 sheep samples were classified as genotype 5. From this aspect, strains found in the Marmara Region as genotypes 3 and 4 were detected only in large ruminants. In the previous studies, it was observed that the strains identified as genotype 4 and 5 were isolated in England, Germany and Greece (Laroucau *et al*., 2009). Genotype 6 to which Greek variant strains belong (Laroucau *et al*., 2009) has not been identified in the Marmara Region.

The regional prevalance of *Chlamydia abortus* in ruminants was shown for the first time in Turkey with Real Time PCR. The results show that *C. abortus* is commonly identified in small ruminants and it is responsible for a significant part of the abortions in the Marmara Region. Therefore, control and measuring strategies including vaccination should be established to decrease the abortion level. There is insufficient data on the status of *C. abortus* infections in humans in Turkey. Considering the prevalence of the disease and the zoonotic potential of the agent (Buxton et al., 2002; Pospischil at al., 2002; Walder et al., 2005; Pichon et al., 2020), some of the human abortion cases may be caused by *C. abortus*. For this reason, *C. abortus* research should be conducted in human abortions with ruminant contact in the Marmara Region.

This study is significant in many aspects. *C. abortus* strains were genotyped for the first time with this study in Turkey. Although the dominant genotype was found to be genotype 2, the existence of genotypes 3, 4 and 5 confirmed the high genotypic diversity of *C. abortus* in the Marmara Region, Turkey. More genotypes can be identified with studies that will be conducted in other regions of the country as well. Secondly, with this study, *C. abortus* was diagnosed for the first time from water buffalo in Turkey. Moreover, *C. abortus* which caused a water buffalo abortion was genotyped with MLVA for the first time in the world, and this work may guide future studies.

Acknowledgements

This work was supported by the Research Fund of Adnan Menderes University (Project No: VTF-18032, PhD Thesis). We would like to thank Merve Engin KURT (Ege University, Faculty of Letters, Department of Translation and Interpreting Studies, Izmir, Turkey) and Mustafa Sencer KARAGUL (Kocaeli University Kartepe Vocational School of Equine Science, Kocaeli, Turkey) for their assistance during the preparation of this manuscript.

References

Aras Z, Sayın Z and Gölen G 2007. Investigation of *Chlamydophila abortus* in abortion of cattle by PCR. Eurasian J Vet Sci. 33(2): 77-80.

Barati S, Moori-Bakhtiari N, Najafabadi MG, Momtaz H and Shokuhizadeh L 2017. The role of zoonotic chlamydial agents in ruminants abortion. Iran J Microbiol. 9(5): 288-294.

Bisias G, Burriel AR, Boutsini S, Kritas S and Leontides LS 2010. A serological investigation of some abortion causes among small ruminant flocks in Greece. Internet J Vet Med. 8(2), DOI: 10.5580/28f4.

Borel N, Thoma R and Spaeni P 2006. Chlamydia-related abortions in cattle from Graubunden, Switzerland. Vet Pathol. 43: 702–708.

Burnard D and Polkinghorne A 2016. Chlamydial infections in wildlife–conservation threats and/or reservoirs of 'spill-over' infections? Vet Microbiol. 196: 78-84.

Buxton D, Anderson IE, Longbottom D, Livingstone M, Wattegedera S and Entrican G 2002. Ovine chlamydial abortion: characterization of the inflammatory immune response in placental tissues. J Comp Pathol. 127: 133–141.

Carter GR and Wise DJ 2004. Essentials of Veterinary Bacteriology and Mycology. 6th ed. Michigan State University Press. East Lansing, Michigan: 290pp.

Chanton-Greutmann H, Thoma R, Corboz L, Borel N and Pospischil A 2002. Abortion in small ruminants in Switzerland: Investigations during two lambing seasons (1996–1998) with special regard to Chlamydial abortions. Schweiz Arch Tierheilk. 144: 483-492.

Ebadi A, Moosakhani F and Jamshidian M 2015. Phylogenetic Analysis of *Chlamydia abortus* Isolated from fetus aborted ewes of Alborz Province. Bull Env Pharmacol. Life Sci. 4: 122-126.

Essig A and Longbottom D 2015. *Chlamydia abortus*. New aspects of infectious abortion in sheep and potential risk for pregnant women. Curr Clin Micro Rpt. 2:22–34.

Galiero G 2007. Causes of infectious abortion in the Mediterranean buffalo. Ital J Anim Sci. 6(2): 194-199.

Givens MD and Marely MSD 2008. Infectious causes of embryonic and fetal mortality. Theriogenology. 10: 10-16.

Greco G, Corrente M, Buonavoglia D, Campanile G, Di Palo R, Martella V, Bellacicco AL, D'Abramo M and Buonavoglia C 2008. Epizootic abortion related to infections by *Chlamydophila abortus* and *Chlamydophila pecorum* in water buffalo (*Bubalus bubalis*). Theriogenology, 69 (9): 1061-1069.

Güler L, Hadimli HH, Erganiş O, Ateş M, Ok U and Gündüz K 2006. Field evaluation of a PCR for the diagnosis of chlamydial abortion in sheep. Vet Rec. 159: 742-745.

Heidari S, Derakhshandeh A, Firouzi R, Ansari-Lari M, Masoudian M and Eraghi V 2017. Molecular detection of *Chlamydophila abortus*, *Coxiella burnetii*, and *Mycoplasma agalactiae* in small ruminants aborted fetuses in southern Iran. Trop Anim Health Prod. 50(4): 779–785.

Kalender H, Kılıç A, Eröksüz H, Muz A, Kılınç U and Taşdemir B 2013. Identification of *Chlamydophila abortus* infection in aborting ewes and goats in Eastern Turkey. Red Med Vet. 164(6): 295-301.

Karagul MS, Malal ME and Akar K 2019. Investigation of *Coxiella burnetii* and *Chlamydia abortus* antibodies in sheep in Düzce region. J DU Health Sci Inst. 9(3): 106-109.

Kılıç K, Kalender H and Muz A 2010. The detection of *Chlamydophila abortus* from aborted bovine fetuses using PCR and microbiological culture. F Ü Sağ Bil Vet Derg. 24(3): 129-132.

Laroucau K, Vorimore F, Bertin C, Mohamad KY, Thierry S, Hermann W, Maingourd C, Pourcel C, Longbottom D, Magnino S, Sachse K, Vretou E and Rodolakis A. 2009. Genotyping of *Chlamydophila abortus* strains by multilocus VNTR analysis. Vet Microbiol. 137: 335–344.

Li Z, Cao X, Fu B, Chao Y, Cai J and Zhou J 2015. Identification and characterization of *Chlamydia abortus* Isolates from yaks in Qinghai, China. Biomed Res Int. doi: 10.1155/2015/658519.

Longbottom D, Fairley S, Chapman S, Psarrou E, Vretou E and Livingstone M 2002. Serological diagnosis of ovine enzootic abortion by enzyme-linked immunosorbent assay with a recombinant protein fragment of the polymorphic outer membrane protein POMP90 of *Chlamydophila abortus*. J Clin Microbiol. 40: 4235–4243.

Longbottom D and Coulter L 2003. Animal Chlamydiosis and zoonotic implications. J Comp Pathol. 128: 217-44.

Merdja SE, Khaled H, Aaziz R, Vorimore F, Bertin C, Dahmani A, Bouyoucef A and Laroucau K 2015. Detection and genotyping of Chlamydia species responsible for reproductive disorders in Algerian small ruminants. Trop Anim Health Prod. 47 (2): 437-43.

Osman KM, Ali HA, ElJakee JA and Galal HM 2012. *Chlamydiaceae* in riverine buffalo (*Bubalus bubalis*) and cows (*Bos taurus*) in Egypt with and without signs of reproductive disease. N Z Vet J. 60(4): 228-233.

Öztürk D, Türütoğlu H and Kaya M 2016. Seroprevalance of *Chlamydophila abortus* infection in goats in Burdur province. MAKU J Health Sci Inst. 1 (2): 17-20.

Pantchev A, Sting R, Bauerfeind R, Tyczka J and Sachse K 2009. New real-time PCR tests for species-specific detection of *Chlamydophila psittaci* and *Chlamydophila abortus* from tissue samples. Vet J. 181: 145, 150.

Pichon N, Guindre L, Laroucau K, Cantaloube M, Nallatamby A and Parreau S 2020. *Chlamydia abortus* in pregnant woman with acute respiratory distress syndrome. Emerg Infect Dis. 26(3): 628–629.

Pospischil A, Thoma R, Hilbe M, Grest P and Gebbers FO. Abortion in woman caused by caprine *Chlamydophila abortus* (*Chlamydia psittaci* serovar 1). Swiss Med Wkly. 2002: 132, 64-66.

Sachse K, Vretou E, Livingstone M, Borel N, Pospischil A and Longbottom D 2009. Recent developments in the laboratory diagnosis of chlamydial infections. Vet Microbiol. 135: 2–21.

Siarkou V, Lambropouros AF, Chrisafi S, Kotsis A and Papadopoulos O. 2002. Subspecies variation in Greek strains of *Chlamydophila abortus*. Vet Microbiol. 85: 145-157.

Siarkou V, Vorimore F, Vicari N, Magnino S, Rodolakis A, Pannekoek Y, Sachse K, Longbottom D and Laroucau K 2015. Diversification and distribution of ruminant *Chlamydia abortus* clones assessed by MLST and MLVA. Plos One, 10 (5): e0126433.

Simeonov K and Chilingirova M 2018. *Chlamydia abortus* and *Coxiella burnetii*- related abortions in small ruminants in Bulgaria during a five-year period (2013-2018). Acta Microbiol Bulg. 34 (4): 236-239.

Szeredi L and Bacsadi À 2002. Detection of *Chlamydophila (Chlamydia) abortus* and *Toxoplasma gondii* in smears from cases of ovine and caprine abortion by the streptavidin-biotin method. J Comp Pathol. 127: 257-263.

Temur A, Dinler U, Seyitoğlu Ş, Kılınç U and Yalçin E 2007. Analysis of *Chlamydia abortus* of cattle reared in Erzurum and surrounding provinces through bacteriological, histopathologic methods. [Online]. Available: https://agris.fao.org/agris-search/search.do?recordID=TR2010000213. Accessed October 22, 2020.

Türütoğlu H and Erganiş O 1996. Studies on isolation of *Chlamydia psittaci* caused to abortion in ewes. J Pen Vet Microbiol. 27: 55-78.

Walder G, Hotzel H, Brezinka C, Gritsch W, Tauber R Würzner R and Ploner F 2005. An unusual cause of sepsis during pregnancy. Obstetrics and Gynecology. 106 (5): 1215–1217.

Ward M 2006. The immunobiology and immunopathology of chlamydial infections. Acta Pathol Microbiol Immunol Scand. 103: 769–796.