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Research Article/Araştırma Makalesi

Seroprevalance of *Chlamydophila abortus* Infections in Goats in Burdur Province

Burdur İlinde Keçilerde Chlamdophila abortus Enfeksiyonunun Seroprevalansı

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Abstract: *Chlamydophila abortus* is the causative agent of enzootic abortion (OEA) in sheep that causes severe economic loss in sheep and goat breeding worldwide. The aim of this study was to detect the seroprevalence of *C. abortus* infection in goat flocks in Burdur province of Turkey. A total of 384 blood serum samples were collected from two years and older goat in randomly selected 22 goat flocks. The apparent and true seroprevalences of individual, within-flock and between- flocks of the *C. abortus* infection were determined in goats by a commercial the enzyme linked immunosorbent assay (ELISA) kit. The apparent and true seroprevalence of *C. abortus* individual, within- flock and between-flocks was calculated as 19.27%, 22.77%, 86.36% and 19.44%, 23.16% and 90.81%, respectively. The seropositivity of *C. abortus* infection to according to flock size were statistically significant ($p<0.05$) between some goat flocks. There was no statistically significant difference between goat breed and *C. abortus* infection ($p>0.05$). In conclusion, these findings showed that *C. abortus* infection is found high rates in goat flocks in Burdur and the control and eradication programs should be started to prevent the spreading of *C. abortus*.

Keywords: *Chlamydophila abortus*, ELISA, Goat, Seroprevalence.

Öz: *Chlamydophila abortus*, tüm dünyada koyun ve keçilerde ciddi ekonomik kayıplara yol açan, koyunların enzootik abortusunun (OEA) etkenidir. Bu çalışmanın amacı, Türkiye'nin Burdur ilinde keçi sürülerinde *C. abortus* enfeksiyonunun seroprevalansını belirlemektir. Toplam 384 kan serum örneği, rastgele seçilen 22 keçi sürüsünde, 2 ve daha büyük yaşlardaki keçilerden toplandı. *C. abortus* enfeksiyonunun bireysel, sürü içi ve sürüler arası seroprevalansı, ticari bir enzyme linked immunosorbent assay (ELISA) kiti ile belirlendi. *C. abortus*'un görünen ve gerçek bireysel, sürü içi ve sürüler arası seroprevalansı sırasıyla 19.27%, 22.77%, 86.36% ve 19.44%, 23.16%, 90.81% olarak hesaplandı. Sürü büyüklüğüne göre *C. abortus* enfeksiyonunun seropozitifliği bazı sürüler arasında istatistiki olarak önemli ($p<0.05$) bulundu. Keçi ırkları ile *C. abortus* enfeksiyonu arasındaki ilişki önemli bulunmadı ($p>0.05$). Sonuç olarak, *C. abortus* enfeksiyonunun Burdur ilinde bulunan keçi sürülerinde yüksek oranda bulunduğu, *C. abortus* enfeksiyonunun yayılımını önlemek için kontrol ve eradikasyon çalışmalarına hemen başlanması gerektiği kanaatine varıldı.

Anahtar Kelimeler: *Chlamydophila abortus,* ELISA, Keçi, Seroprevalans.

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Introduction

Ovine enzootic abortion (OEA) is a chronic disease caused by *Chlamydophila abortus* (*C. abortus*). *C. abortus* is a compulsory intracellular bacterium that causes abortion, stillbirth and poor offspring in small ruminants. *C. abortus* leads to significant economic losses in sheep and goat flocks in all over the world (Aydın and Paracıkoğlu, 2006). *C. abortus* causes usually abortions in 2-3 weeks before birth (Kalender et al., 2013). Subclinical infection can be transmitted to healthy animals and flocks through the placenta, vaginal discharge and aborted fetus of infected animals (Kalender et al., 2013). *C. abortus* is a zoonotic agent that can cause infections in humans, too. *C. abortus* can cause abortion in pregnant women in close contact with aborted sheep and goats (Kerr et al., 2005; Pospischil 2006). In addition, breeders, veterinarians, slaughterhouse workers, workers in

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the vaccine production and laboratory workers can be infected by inhalation, taking into account the effects of urine, fecal and fetal fluids of infected animals (Hadley et al., 1992; Cislakova et al., 2007; Ortega et al., 2016).

The diagnosis of infection is made by direct and indirect diagnostic methods. Direct diagnostic methods are intended to demonstrate the presence of the agent. The isolation is the gold standard (Cantekin et al., 2015; Essig and Longbottom, 2015). But, *C. abortus* is a compulsory intracellular bacterium and it can not breed in the media, so isolation only involves culturing clinical specimens in laboratory animals, or tissue cultures or embriyonated chicken eggs (Aydın and Paracıkoğlu 2006; Cantekin et al., 2015). The isolation is not routinely performed because of the time consuming as well as the expert staff (Cantekin et al., 2015; OIE 2012, Beckman, 2019). The serologic tests are often used in diagnosis of infection. Complement fixation test (CFT) is a test used in the diagnosis of *C. abortus* infection and recommended by OIE (2012). But, cross reaction is detected between Gram negative bacteria such as *C. abortus*, *C. pecorum* and *Acinetobacter* sp. is low the sensitivity and specificity of test (OIE 2012). ELISA, a more sensitive and specific test than CFT, is frequently used in the detection of *C. abortus* infection in field and experimental studies (Vlahovic et al., 2001; Longbottom and Coulter, 2003). Its application is easy, cheap and at the same time testing a large number of animals and getting the results in a short time is the biggest advantage (Villagro-Blanco et al., 2015, OIE 2012).

The seroprevalence of *C. abortus* infection in sheep has been reported 1.81 %- 32 % in Turkey (Duman and Durak, 1998; Baz and Aydın, 2006; Caya et al., 2006; Küçükkayan et al., 2007; Otlu et al., 2007; Öztürk et al., 2016). In Burdur, there are two studies on the prevalence of *C. abortus* infection in sheep and cattle using ELISA (Öztürk et al., 2012; Öztürk et al., 2016). In these studies, *C. abortus* infection could not detectable in cattle, but the prevalence of individual, within-flocks and between flocks in sheep were 32%, 40% and 80%, respectively. Although presence of *C. abortus* infection in goats has known, the seroprevalence of *C. abortus* in goats had not been investigated in Turkey before.

The aim of the present study was to determine the apparent and true prevalence of *C. abortus* infection in goat flocks in Burdur province of Turkey.

Materials and Methods

Sampling

This study was conducted in between October 2016 and January 2017. The blood serum samples were collected from 22 goat flocks (Hair goat:12, Honamlı goat: 10) found least 20 goat with aged 2 years and older female goats, according to records of the Burdur Association of Sheep and Goat Breeders. The size of the flocks were changed between 40-500 animals. The goats were selected with random sampling method from flocks. The flock information used in this study was given in Table 1. Burdur province, which is located in southwest of Turkey is approximately 7,135 km^2 and a crossing area between Aegean, Middle Anatolia and Mediterranean parts of Turkey. The mean altitude is 1000 m above sea level.

In order to determine the prevalence rate without error, the sample size was determined according to epidemiological criteria. Since there was no study on seroprevalence of *C. abortus* in goats in Burdur province of Turkey, the estimated prevalence was accepted as 50% (Erganiş and Uçan, 2001). The minimum number of samples to be used in the survey according to 95% confidence interval and 5% error margin was determined as 384 (Erganiş and Uçan 2001). The 384 serum samples were collected from 11 to 20 goats in each flocks between October 2016 and January 2017. Blood samples were collected in 10 ml vacutainer tubes from the juguler vein of goats. Blood samples were transported to Burdur Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Microbiology Laboratory. The samples were centrifuged for 5 minutes at 5000 x

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g and the serum samples were stored at -20°C until using.

ELISA

The serum samples were tested for *C. abortus* antibodies using a commercial ELISA kit (IDEXX, Switzerland, Liebefeld-Bern, Switzerland) according to the manufacturer's instructions. The absorbance value (OD) of each well was read in a 450 nm ELISA reader (Microplate Reader RT-2100C, Rayto and Analytical Sciences Co Ltd, PRC). The mean OD values of the positive (PC) and negative controls (NC) were calculated. According to the kit protocol, the test was accepted as valid if the mean value of PC (PCx) was ≤ 2,000, the mean value of NC (NCx) was ≤0,500 and PCx-NCx ≥ 0,300. The ratio S/P (% S / P) for each sera sample was calculated according to the following formula. Example P / S % = Example- NCx- / PCx-NCx- X 100. The S/P ratios of sera samples were evaluated as negative ≤ 30%, 30 % - 40 % suspicious, and ≥ 40 % positive for *C. abortus* antibodies. The flocks in which at least one seropositive animals for *C. abortus* were accepted to "positive flocks".

Table 1. The villages according to numbers of samples and herd size in this study

	Villages	Number of blood samples	Herd sizes
1	Kayis/Central village	20	430
2	Kayis/ Central village	20	160
3	Kayis /Central village	20	300
4	Kayis /Central village	20	350
5	Cine /Central village	12	100
6	Tas kapi/ Central village	20	40
7	MAKU goat farm /Central village	20	250
8	Guneyyayla /Central village	10	40
9	Guneyyayla /Central village	10	40
10	Guneyyayla /Central village	11	45
11	Yarisli /Yesilova	20	230
12	Kayadibi /Yesilova	20	205
13	Kayadibi /Yesilova	20	230
14	Harmanli/Yesilova	20	300
15	Kartalpinar	20	164
16	Bolmepinar/Cavdir	20	300
17	Cavdir	18	500
18	Kizillar / Cavdir	20	350
19	Bayir / Cavdir	19	430
20	Bayir / Cavdir	12	250
21	Karakoy / Cavdir	14	212
22	Cavdir	18	170
	Total	**384**	**5096**

Calculation of appearent prevalence

The appearent individual, within-flock and between- flock prevalence was calculated in 95% confident interval (GA) according to the Wilson binominol estimation method (Brown et al., 2001).

Calculation of true prevalence

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The true individual, within- flock and between-flock prevalence was calculated according to the Rogan-Gladen estimation method (Rogan and Gladen, 1978). In the calculations, Idexx Chlamydiosis Total Ab Test kit manufacturer (IDEXX) was used in 95% sensitivity and 99% specificity which is reported for the goats.

Statistical analysis

First, the data were entered in SPSS computer statistical program and whether serum samples collected from the flocks showed normal distribution was determined by One Sample Kolmogorov-Smirnov test. The association between the seroprevalence of *C. abortus* infection and flock size were calculated using the Chi-square test (χ^2-test). The relation between the flock size and the seroprevalence of infection rate was tested by simple linear regression.

Questionnairs

A questionnaire was carried out with goat owners (flock size, age, breeding and reproductive disorders such as stillbirths and abortions. The village names, sample numbers, size of flock was given in Table 1-2.

Table 2. Distrubition of *C. abortus* seroprevalances in goat herds

Number of herds (N=22)				Number of sample (N=384)			
Positive		Negative		Positive		Negative	
N	%	N	%	N	%	N	%
19	86.36	3	13.64	74	19.27	310	80.73

N: Number of animals

Results

Questionnaire results:

In this study, the serum samples were collected from Hair (n:12) and Honamli (n:10) goat flocks. The age of goats were no known, but blood samples were collected from 384 goat older than 2 years. The flock owners reported that 16 out of 22 flocks had abortions and died within a few days after birth of kids in 15 flocks.

ELISA results

The seroprevalence for *C. abortus* infection in goats were determined to be 19.27 % (74/384) (Table 3). The seropositivity was detected in 19 of 22 goat flocks. The seroprevalence of infection were changed from 5% to 60% in the positive goat flocks (Table 2 and 3). While only five of the positive flocks had one seropositive animal and fourteen flocks had two or more positive animals (Table 3). The seropositivity for *C. abortus* was determined in 4 of the 5 flocks that had no reproductive problems.

In this study, the serum samples were collected from Hair and Honamli goat flocks. Seropositivity for *C. abortus* in Hair and Honamli goats were detected in 15.5 % and 23.34%, respectively (Table 4). The relationship between goat breed and *C. abortus* infection was evaluated statistically.

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Table 3. Sizes, number of aborted fetus and stillbirth and ELISA results in goat herds for *C. abortus*.

Herds no	Herd sizes	Aborted goat		Stillbirth		ELISA positive		ELISA negative		Number of samples
	N	N	%	N	%	N	%	N	%	
1	430	35	8.14	11	2.6	3	15	17	85	20
2	160	10	6.25	20	6.3	1	5	19	95	20
3.	300	10	3.33	20	6.7	1	5	19	95	20
4.	350	20	5.71	10	2.9	5	20	15	80	20
5.	100	15	15	15	15	6	50	6	50	12
6.	40	5	12.5	6	15	2	10	18	90	20
7.	250	0	0	0	0	3	15	17	85	20
8.	40	0	0	0	0	2	20	8	80	10
9.	40	0	0	1	2.5	5	50	5	50	10
10.	45	3	6.7	2	4.4	6	54.55	5	55.45	11
11.	230	0	0	0	0	3	15	17	85	20
12.	205	4	1.9	1	0.5	12	60	8	40	20
13.	300	20	6.7	15	5	2	10	18	90	20
14.	230	6	2.6	10	4.3	11	55	9	45	20
15.	164	2	1.22	0	0	0	0	20	100	20
16.	300	15	5	10	3.3	4	20	16	80	20
17.	500	0	0	0	0	1	5.55	17	94.45	18
18.	350	10	2.9	2	0.6	0	0	20	100	20
19.	430	0	0	0	0	0	0	19	100	19
20.	212	50	23.6	35	16.5	1	7.14	13	92.86	14
21.	170	50	29.4	0	0	1	5.56	17	94.44	18
22.	250	50	20	10	4	5	41.67	7	58.33	12
Total	**5096**	**295**	**5.8**	**168**	**3.3**	**74**	**19.27**	**310**	**80.73**	**384**

N: Number of animals

Table 4. Distribution of *C. abortus* seroprevalence in goats

Goat breed	*C. abortus* positive	
	N	%
Hair goat (N:200)	31	15.5
Honamlı goat (N:184)	43	23.34
Total	**74**	**38.84**

N: The number of animals

Apparent and true prevalence results

In this study, the apparent the individual, within-flock and between- flocks prevalence values for *C. abortus* infection in the goats were calculated to be 19.27% (95% GA: 15.64% -23.51%), 22.77% (95% GA: 18.54-27.63%) and 86.36% 95% GA: 66.67% -95.25%), respectively (Table 5). By the spesificity and sensitivity of *C. abortus* ELISA kit, the true individual, within-flock and between-flock prevalence values for *C. abortus* infection were detected 19.44%(95%GA:15.24-23.63), 23.16%(95% GA:18.31-28.01), 90.81% (95% GA: %75.56-106.07), respectively (Table 5).

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Table 5. The prevalance apparent and true individual, within-herd and between-herd in goats.

			Apparent prevalence		True prevalence	
			Estimated (%)	**% 95 GA**	**Estimated (%)**	**% 95 GA**
Prevalence	Samples	Positive				
Individual	384	74	19.27	15.64-23.51	19.44	15.24-23.63
Within-herd	335	74	22.09	17.98-26.83	22.44	17.71-27.16
Between-herd	22	19	86.36	66.67-95.25	90.81	75.56-106.07

Statistical analysis

The highest prevalence of *C. abortus* infection was 29% in the flock had 120-239 aminals. The lowest prevalence of infection was 9% in the largest flock size group that had over 340 animals. The difference (P<0.05) between the values carrying different letters in the same column was found significant (Table 6).

The range of seropositivity for *C. abortus* was showed differences in goat flocks (Table 4). The difference in the prevalence of *C. abortus* infection between the goat breed was not statistically significant (P>0.05).

Table 6. Seroprevalence of *C. abortus* infection in goats according to flock size in Burdur province.

Size of flock	Number of flocks	Number tested	Seropositives (%)	Rate of seropositive (%)
40-119	5	63	21[a]	10-54.55
120-249	7	132	29[ac]	0-60
250-339	5	92	15[bc]	5-41.67
340-500	5	97	9[b]	0-20
Total	21	384	19.27	0-60

*P<0.05: The difference (P<0.05) between the values carrying different letters in the same column was found significant.

Discussion

Chlamydophila abortus is responsible for abortion, infertility, keratoconjunctivitis, pneumonia, enteritis, mastitis and arthritis in ruminants (Reinhold et al., 2011). The seroprevalence of *C. abortus* infection have been usually investigated in sheep in Turkey (Baz and Aydın, 2006; Duman 1996; Caya et al., 2006; Gokce et al., 2007; Otlu et al., 2007; Muz et al., 2014). Although the presence of *C. abortus* infection has been known for a long time in goats of Turkey (Kalender et al., 2013) there are no studies to determine the seroprevalence of *C. abortus* infection in goats.

In this study, the flock owners reported that 16 out of 22 flocks had abortions and died within a few days after birth of kids in 15 flocks. Out of 22 flocks, 19 were positive for *C. abortus* antibodies. The abortion was detected in 2 out of 3 negative flocks for *C. abortus* antibodies. In Turkey, the high prevalence of abortion cases in small ruminants is due to generally brucellosis (Arda et

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al., 1987; Küçükkayan et al., 2007; Otlu et al., 2007). But, the etiological agents as *C. burnetii*, *T. gondii*, *Leptospira* sp., *Listeria monocytogenes* may cause abortion in small ruminants, too (Arda et al., 1987; Otlu et al., 2007). In this study, the seropositivity were detected in 14 of the flocks with abortion problem and all of the flocks with kid death. ELISA was positive in 4 goat flocks that were considered to be healthy and no reproductive problem such as kid death and abortion. The goat owners were reported that animal movements were high in farms, the animals were taken from random flocks, and history of animals and flocks were unknown. The researchers (Aydın and Paracıkoğlu, 2006) reported that once aborted animals did not abort a second time. However, the animals infected during the late stages of pregnancy can not usually abort, but can develop abortion in next season (Aydın and Paracıkoğlu, 2006).

In this study, the seroprevalence of *C. abortus* infection ranged from 5% to 60% in goat flocks. The highest prevalence of *C. abortus* infection was 29% in the flock had 120-239 aminals. The lowest prevalence of infection was 9% in the largest flock size group that had over 340 animals. However, there was no statistically significant difference between goat breed and *C. abortus* infection ($p>0.05$). Al Qudah et al. (2004) reported that there was correlation between the seroprevalence of *C. abortus* infection and flock size in sheep flocks, but there was no significant correlation in goat flocks. In this study, the apparent and true prevalence of within-flock of *C. abortus* infection was determined 22.77% and 23.16% in goat flocks, respectively. The apparent prevalence of within-flock of *C. abortus* infection were reported between 11.37%-52.9% in goat flocks (Al-Qudah et al., 2004; Masala et al., 2005; Hernandez et al., 2014, Yin et al., 2014). Hernandez et al. (2014) reported that the prevalence of *C. abortus* infection was 4.87% in goats and ranged from 3.44% to 13.51% in flocks. This rate was mostly low from the present study results. This results can be originated from grown together goat and sheep in the same flocks in Burdur province. The researchers (Aydin and Paracikoglu, 2006; Quinn et al., 2009) reported that the sheep can be source of *C. abortus* infection in goat. In the present study, while the apparent prevalence of individual, within flocks and between- flocks for *C. abortus* infection was calculated as 19.27%, 22.09% and 70.37%, respectively, true prevalance of *C. abortus* individual, within- flocks and between- flocks was detected 19.44%, 22.44% and 90.81%, respectively. When these results were compared with the study done in sheep of Burdur province, the prevalence of individual, within-flock and between- flocks the seroprevalence of *C. abortus* in goats was found low. The sheep are more susceptible to *C. abortus* infection than the goats (Quinn et al., 2009). In Burdur, goats and sheep are generally grown together in the same flocks. It can be possible that infections are transmitted from sheep to goats. Furthermore, the high seroprevalence of the infection may be due to the lack of studies on protection and control of *C. abortus* infection.

In this study, the apparent and true prevalence of individual for *C. abortus* infection was calculated as 19.27% and 19.44% respectively. The prevalence of *C. abortus* in goats has been investigated in other countries by different serological tests (20, 48, 54, 82, 83, 94, 97, 101, 104). A Belgian study reported that the seroprevalence of *C. abortus* infection in goats was 18.75% (Yin et al., 2014). The seroprevalence of *C. abortus* infection was reported % 21.2 in Greece (Bisias et al., 2009), 25.6% in Iran (Esmaeili et al., 2015). These results were similar to our study results. But, our study results were found lower than that Taiwan (Wang et al., 2001) and Bosnia-Herzegovina (Krkalic et al., 2015). Our study was conducted in random herds, while both studies were conducted in flocks with abortion problems. While the seroprevalence of *C. abortus* infection in goats was found to be 0.22% in Poland (Czopowicz et al., 2010), 5.8% in Italy (Masala et al., 2005), 7.7% in Slovakia (Cislakova et al., 2007), 11.4% in Jordan (Al-Oudah et al., 2004), 9.3% in Brasil (Santos et al., 2012), 4.87% in Mexico (Hernandez et al., 2014), we was detected 19.27%. The cause of this differences can be differences in

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management of herds, climates, coexistence of sheep and goats, different detection methods, uncontrolled animal moviments and the lack of studies on protection and control of *C. abortus* infection.

In our study, the apparent and true prevalence of between flocks of *C. abortus* infection were 86.36%-90.81%, respectively. Yin et al. (2014) reported that the apparent prevalence of between flocks of the infection was 11.11%, while the apparent prevalence of between flocks of *C. abortus* infection in goats was 48.4% by Masala et al. (2015) and 100% by Al-Quadah et al. (2004). Samkange et al. (2010) reported that the prevalance of between flocks was changed between 17.2%-54%, in goat flocks. However, Czopowicz et al. (2010) was detected 4.2%. In the present study, the prevalence of between flocks of *C. abortus* infection was higher than other studies, while the prevalence of between flocks of *C. abortus* infection was lower than Al-Quadah (2004)'s study. The differences for *C. abortus* infection rate can be probably due to the different diagnostic methods, management, uncontrolled animal movements and it may vary depending on the region where the study was conducted (Rekiki et al., 2002; Masala et al., 2005; Güler et al., 2006; Kalender et al., 2013; Osman, 2013).

In conclusion, In Burdur, the apparent and true prevalence of individual, within-flock and between- flocks seroprevalence of *C. abortus* infection was higher than the other countries. In this context; a control program for *C. abortus* infection should be planned goat breeding in Turkey

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