Kafkas Univ Vet Fak Derg
26 (6): 777-785, 2020
DOI: 10.9775/kvfd.2020.24380

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Accomplished Management of *Chlamydophila abortus*-induced Enzootic Sheep Abortions: The Case of Şavşat (Turkey)

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Article ID: KVFD-2020-24380 Received: 30.04.2020 Accepted: 09.09.2020 Published Online: 15.09.2020

How to Cite This Article

Büyük F, Özgen EK, Karakurt E, Coşkun MR, Büyük E, Özmen M, Dağ S, Çelik E, Gülmez Sağlam A, Karadeniz Pütür E, Ulucan M, Nuhoğlu H, Şahin M: Accomplished management of *Chlamydophila abortus*-induced enzootic sheep Abortions: The case of Şavşat (Turkey). *Kafkas Univ Vet Fak Derg,* 26 (6): 777-785, 2020. DOI: 10.9775/kvfd.2020.24380

Abstract

Infectious sheep abortions caused by bacterial agents such as *Brucella melitensis, Campylobacter* spp., *Listeria* spp., *Chlamydophila abortus* etc. lead significant economic losses in sheep enterprises. Many of these bacteria such as *C. abortus* that causes enzootic sheep abortion are contagious and zoonotic, as well. "Good veterinary practices" performed accurately and timely are extremely important in the management of the outbreak and minimizes the economic losses caused by these infections. This study aimed to diagnose infectious sheep abortions and to manage the outbreaks observed in two enterprises with 850 Hemşin sheep in Şavşat district of Artvin province, Turkey. The disease was diagnosed by conventional and real-time PCRs with detecting *C. abortus* DNA in five aborted fetal tissues. The diagnosis was confirmed immunohistochemically. In the course of the outbreak management, aborted sheep were weed out, treated with 5 mL/sheep I.M. of oxytetracycline for 5 times 24 h apart and got maintained for 3-4 weeks until disposed of by the owners. Pregnant sheep were administered 5 mL/sheep I.M. of oxytetracycline for 3 times as 3 days after the first application and 5 days after the second application. A mineral-amino acid supplement was also administered to the pregnant sheep by adding 15 mL of the drug to 0.5 L water for each sheep for 3 days. For the prophylactic purpose, tetracycline with a dose of 20 mg/kg bw/day was recommended to add to drinking water once a day for 3-5 days following the initial treatment. For biosafety, aborted materials were covered with quicklime and buried in soil depth of 1.5 m and contaminated areas were disinfected with 0.5% bleach once a day for 3 days. A protective immunization could not be done because of the absence of vaccines in the national market and some concerns about the vaccination of late-pregnant sheep. The sheep enterprises were closely monitored for 4-5 weeks until the birth season ended.

Keywords: *Chlamydophila abortus, Hemşin sheep, Outbreak management, Şavşat, Artvin, Turkey*

Chlamydophila abortus Kaynaklı Enzootik Koyun Abortlarının Başarılı Yönetimi: Şavşat Örneği (Türkiye)

Öz

Brucella melitensis, Campylobacter spp., *Listeria* spp., *Chlamydophila abortus* gibi bakteriyel ajanların neden olduğu infeksiyöz koyun abortları, koyun işletmelerinde önemli ekonomik kayıplara yol açarlar. Enzootik koyun abortlarına neden olan *C. abortus* ve sayılan diğer bakterilerin çoğu aynı zamanda bulaşıcı ve zoonotiktir. Doğru ve zamanında yapılan "iyi veteriner hekimlik" uygulamaları salgının idaresinde son derece önemli olup enfeksiyon kaynaklı ekonomik kayıpları minimum seviyeye indirmektedir. Bu çalışmada, Artvin ili Şavşat ilçesinde 850 Hemşin koyunu bulunan iki işletmede gözlenen infeksiyöz koyun abortlarının teşhis edilmesi ve salgının idaresi amaçlanmıştır. Hastalık, atık beş kuzuya ait fötal dokularda *C. abortus* DNA'sının konvansiyonel ve real-time PCR'ler ile saptanması ile teşhis edilmiştir. Teşhis, immunohistokimyasal yöntemlerle doğrulanmıştır. Salgın yönetimi kapsamında, atık yapan koyunlar ayıklanmış, 5 mL/koyun dozda oksitetrasiklin ile İ.M. yolla 24 saat arayla 5 defa sağaltılmış ve elden çıkarılıncaya kadar 3-4 hafta süresince beslenmiştir. Gebe koyunlara, ilk uygulama, 3 gün sonra ikinci uygulama ve 5 gün sonra üçüncü uygulama şeklinde İ.M. yolla 5 mL/koyun dozda oksitetrasiklin uygulanmıştır. Ayrıca bu hayvanlara 3 gün süresince 0.5 L suya 15 mL katılmak üzere aminoasit-mineral takviyesi de yapılmıştır. İlk ilaç uygulamasını takiben profilaktik amaçlı, 20 mg/kg c.a./gün dozda tetrasiklinin günde bir kez olmak üzere 3-5 gün süreyle içme sularına katılması tavsiye edilmiştir. Biyogüvenlik kapsamında, atık materyalleri sönmemiş kireçle kaplanarak toprağa 1.5 m derinlikte gömülmüş ve kontamine alanlar %0.5'lik çamaşır suyu ile günde bir kez olmak üzere 3 gün süreyle dezenfekte edilmiştir. Ulusal pazarda aşının olmayışı ve ileri gebe koyunlarda aşı uygulanması ile ilgili bazı endişelerden dolayı koruyucu bir aşılama yapılamamıştır. Koyun sürüleri doğum sezonu bitene kadar 4-5 hafta süreyle yakından takip edilmiştir.

Anahtar sözcükler: *Chlamydophila abortus, Hemşin koyunu, Salgın yönetimi, Şavşat, Artvin*

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INTRODUCTION

Main problems encountered in the sheep raising are perinatal lamb deaths and pneumonia. Among the causes of perinatal lamb deaths, the most prominent are infectious abortions and diarrhea. It is reported that a significant part of sheep abortions in Turkey has an infectious character. Infectious sheep abortions are caused by bacterial, viral and paracetic agents such as Brucellosis, Campylobacteriosis, Chlamydiosis, Listeriosis, Salmonellosis, Bluetongue Virus (BTV), Bovine Viral Diarrhea-Mucosal Disease (BVD-MD), Akabane and *Toxoplasma gondii* [1-4]. Among these, Chlamydial abortions are the infections caused by *Chlamydophila abortus (Chlamydia psittaci*-serotype 1) and lead to reproductive deficiencies, enzootic abortions and related economic losses in sheep [5,6]. Indeed, it has been estimated that Chlamydial agents cause an annual economic losse of 11-48 million pounds in sheep abortions observed in England [7]. Although the prevalence of Chlamydial abortions varies by country, the rate can reach 30% in naive herds. Chlamydial agents are scattered around through the vaginal discharges of aborted sheep and tissues of aborted fetus and transmitted by oral route following the oronasal contact of susceptible animals. The main clinical symptom of *C. abortus* infection is abortion observed in the last 2 or 3 weeks of gestation, which is emerged following an inflammation, thickness and vascular thrombosis in placenta. The abortion can sometimes be seen as an abortion storm, where too many sheep deliver the fetus involuntarily in a short time [8].

As the clinical findings are similar in many infections with abortion, the exact diagnosis of *C. abortus* is made by laboratory analysis. For this purpose, imaging of the elementary bodies on direct smears is agent-specific, however, definitive diagnosis is required an *in vivo* isolation or PCR confirmation of the agent. Immunohistochemical analysis of sections taken from fresh fetal tissues also provides useful information in the diagnosis. Although antibody titer increased 2-3 weeks after the abortion can be detected by Complement Fixation Test (CFT) and ELISA, the possibility of cross-reaction should be considered [9]. In the course of active Chlamydial infection, all animals in the herd, including pregnant sheep, can be treated with long-acting oxytetracycline. On the disease control, the main outbreak management practices that can be applied are early detection of abortion cases, screening of whole herd, isolation of aborted animals, disinfection of contaminated areas and vaccination of healthy animals [10,11].

This study is based on the diagnosis of infectious agents and the management of the enzootic sheep abortions observed in two enterprises where intensive sheep breeding is carried out in Şavşat district of Artvin province.

MATERIAL and METHODS

The study was carried out in two intensive sheep enterprises suffered from infectious abortions in Şavşat district Artvin province (Turkey), in February of 2020. The ethical permission of the study was ensured by the decision of The Kafkas University Animal Experiments Local Ethics Committee with the code of "KAÜ-HADYEK/2020-064". Study material composed of brain, liver, lung, kidney, spleen and stomach (abomasum) contents of five aborted fetuses was obtained from one of the enterprises. As the storage of aborted materials belonging to the other enterprise is not properly maintained, no sample could taken for laboratory diagnosis. All samples were taken following the systemic necropsy of aborted fetuses and maintained properly. For bacterial isolation, approximately 5 g of each fetal tissue and 1 mL of abomasum contents were kept in sterile containers. For molecular analysis, 25-50 mg fetal tissues and 100 µL abomasum content were homogenized in MagNA Lyser Green Beads tubes (Roche, Switzerland). For immunohistochemical analysis, the samples were maintained in 10% buffered formaldehyde solution. All reagents and chemicals were obtained from Fisher Scientific (Loughborough, UK) or Sigma-Aldrich (Dorset, UK) unless otherwise stated in the text.

Cultural Analysis

Cultural analyses were carried out in Microbiology Laboratories of Veterinary Faculty in the Kafkas University and in Erzurum Veterinary Control Institute. *Brucella* spp., *Salmonella* spp. and *Campylobacter* spp. analyzes were done by cultural methods from the samples. For *Brucella* spp. isolation, the samples were plated on Farrell medium plates and incubated at 37°C in microaerophilic conditions for 8 days. Presence of the typical honey-colored smooth colonies with a diameter of 1-2 mm was evaluated on the medium [12]. *Campylobacter* spp. isolation from the samples was done on Skirrow medium. The plated media were incubated at 37°C in microaerophilic condition for 5 days and evaluated for the typical colony presence [13,14]. *Salmonella* spp. isolation from the samples was performed on XLD medium. The plated media were incubated at 37°C in aerobic environment for 5 days and evaluated for the typical colony morphology [15]. Moreover, the fetal tissue samples were plated on 7% sheep blood agar plates and incubated at 37°C for 72 h in aerobic and microaerobic conditions and evaluated for the other bacterial abortive agents which could be culturable *in vitro* conditions.

Molecular Analysis

Molecular analyses were carried out in Molecular Microbiology Laboratories of Veterinary Faculty in the Kafkas University and in Erzurum Veterinary Control Institute.

Conventional PCR and real-time PCR techniques were used for direct diagnosis of the abortive agents. In this context, analysis of bacterial abortive agents such as *C. abortus* and *Leptospira* spp., parasitic agents such as *T. gondii* and viral agents such as Bluetongue Virus (BTV), Small Ruminant

Plague (PPR), Akabane and Bovine Viral Diarrhea (BDV) Virus were performed.

Nucleic acid extraction: Total nucleic acid was extracted through an automated extraction device (Qiacube, Qiagene, Germany) and a commercial kit (Qiamp cador pathogen mini kit, Qiagen, Germany). Tissue samples were placed into sterile homogenization tubes containing 500 µL of PBS, and then lysed at 6000 rpm for 1 min in MagNa Lyser© Instrument (Roche, Switzerland). Tissue samples were then centrifuged at 6000 rpm for 3 min and 200 µL of supernatant was used for extraction.

Conventional PCR analysis: Conventional PCR analysis of *C. abortus* was performed with primers which amplify the polymorphic membrane protein *(pmp)* gene of the bacteria [16] *(Table 1)*. Reaction volume was formed from 5 µL PCR buffer, 0.4 µL dNTP mix, 0.3 µL primer-F, 0.3 µL primer-R, 0.4 µL Taq DNA polymerase, 15.6 µL nuclease-free water and 3 µL template DNA. Cycling conditions were consisted of 5 min of initial denaturation at 94°C, followed by 30 cycles of 30 sec of denaturation at 94°C, 60 sec of annealing at 50°C, and 120 sec of extension at 72°C. The PCR was finalized with an extension at 72°C for 10 min. Amplified products were analyzed by 1.5% agarose gel electrophoresis and 300 bp size products were evaluated.

Real-time PCR analysis: Analysis of bacterial and parasitic agents was performed by LightCycler® Taqman® Master kit (Roche, Switzerland) with using the primers and probes [17-19] specified in *Table 1*. Reaction volume was composed of 9 µL nuclease-free water, 0.5 µL forward primer (10 µM), 0.5 µL reverse primer (10 µM), 4 µL FastStart mix, 1 µL Taqman probe (4 µM) and 5 µL template DNA. PCR amplification was performed on a real-time PCR machine (LightCycler® 480 Instrument II, Roche, Switzerland). Cycling conditions were consisted of 10 min of initial denaturation at 95°C, followed by 45 cycles of 3 sec of denaturation at 95°C, 30 sec of annealing at 60°C, and 1 sec of extension at 72°C.

Analysis of viral agents was done with one step real-time RT-PCR method. For this purpose, QuantinNova Pathogen + IC Kit (Qiagen, Germany) was used. Reactions were carried out in the presence of primers and probes [20-24] specified in *Table 1*. Nucleic acid extracts were used after 3 min denaturation at 95°C in order to denaturate RNA of the double-stranded BTV. RT-PCR reaction in 20 µL volume was composed of 6 µL nuclease-free water, 5 µL RT-PCR master mix, 2 µL probe assay, 1 µL IC RNA, 0.5 µL forward primer (10 µM), 0.5 µL reverse primer (10 µM), 1 µL probe (4 µM) and 4 µL template RNA. cDNA acquisition and subsequent amplification were performed on the real-time PCR machine. The one step real-time RT-PCR thermal

Table 1. *Primer and probe sequences used for real-time PCR*

The Agent	Primer and Prob Sequences (5'–3')		References
C. abortus pmp gene	Forward	ATGAAACATCCAGTCTACTGG	[16]
	Reverse	TTGTGTAGTAATATTATCAAA	
Leptospira spp.	Forward	AAGCATTACCGCTTGTGGTG	[17]
	Reverse	GAACTCCCATTTCAGCGATT	
	Probe (FAM/BHQ1)	AAAGCCAGGACAAGCGCCG	
T. gondii B1 gene	Forward	GGAATGAAAGAGACGCTAATGTGTT	[18]
	Reverse	ACAGATACTCATGAATTTCACTTTTCG	
	Probe (FAM/TAMRA)	TTGCAGTCACTGACGAGCTCCCCTCT	
C. abortus ompA gene	Forward	GCAACTGACACTAAGTCGGCTACA	[19]
	Reverse	ACAAGCATGTTCAATCGATAAGAGA	
	Probe (FAM/TAMRA)	TAAATACCACGAATGGCAAGTTGGTTTAGCG	
AKABANE	Forward	GCTAGAGTCTTCTTCCTCAACCAGAA	[20]
	Reverse	AAAAGTAAGATCGACACTTGGTTGTG	
	Probe (FAM/TAMRA)	CCAAGATGGTCTTACATAAGAC	
BVD	Forward	CCATRCCCDTAGTAGGACTAGC	[21,22]
	Reverse	GYGTYGAACTACTGACGACT	
	Probe (FAM/BHQ1)	ACTAGCCGTCGTGGTGAAATCCCTGAGTGG	
BTV	Forward	TGGAYAAAGCRATGTCAAA	[23]
	Reverse	ACRTCATCACGAAACGCTTC	
	Probe (FAM/TAMRA)	ARGCTGCATTCGCATCGTACGC	
PPR	Forward	CACAGCAGAGGAAGCCCAACT	[24]
	Reverse	TGTTTTGTGCTGGAGGAAGGA	
	Probe (FAM/TAMRA)	CTCGGAAATCGCCTCGCAGGC T	

cycle was initiated with a reverse transcriptase step at 50°C for 10 min. The reaction was continued with an initial denaturation at 95°C for 2 min, followed by an amplification step of 45 cycles at 95°C for 5 sec of denaturation and 60°C for 30 sec of annealing.

Immunohistochemical Analysis

Immunohistochemical analyses were carried out in Pathology Laboratories of Veterinary Faculty in the Kafkas University.

Hematoxylin-Eosin staining: Following the systemic necropsy of aborted fetuses, tissue samples (lung, liver) were fixed in 10% buffered formaldehyde solution. After the routine tissue follow-up, sections at 5 µm were taken from paraffin blocks prepared for Hematoxylin & Eosin (H&E) staining. The tissue sections were examined and photographed (Olympus Soft Imaging Solutions GmbH, 3,4, Olympus, Germany) under a light microscope (Olympus, Germany) to detect histopathological changes.

Immunohistochemical staining: Avidin-Biotin Peroxidase method was used as an immunohistochemical method [25]. For this purpose, the sections at 4 µm taken from paraffin blocks were rehydrated. The sections were treated with 3% Hydrogen peroxide solution for 15 min to prevent endogenous peroxidase activity. The microwave method was then applied (Citrat Buffer Solution, pH: 6, for 25 min) to the sections to reveal antigenic receptors. The sections were incubated with non-immune serum (Genemed Biotechnologies, Germany) for 30 min to prevent non-specific staining. Subsequently, the sections were incubated for 1 h at room temperature with an anti-Chlamydia primary antibody (Progen, Germany) diluted 1/100 in Phosphate Buffered Salt Solution (PBS). The sections were washed 3 times for 5 min in PBS solution, and subjected to biotinylated antibody (Genemed Biotechnologies, Germany) for 30 min at room temperature. After washing in PBS for 3-5 min, all the sections were incubated for 30 min with peroxidase-linked Strep Avidin (Genemed Biotechnologies, Germany). 3.3'-Diaminobenzidine tetrahydrochloride (DAB) (Genemed Biotechnologies, Germany) solution was used as the color-producing substrate. The sections were stained with Mayer's Hematoxylin and covered with immune mount. The smears prepared after the covering were examined under a light microscope (Olympus, Germany) and photographed.

Outbreak Management Practices

Outbreak management practices [10,11] were carried out in two different sheep farms, which suffered from the outbreak in the same period and included a total of 850 Hemşin sheep. In this context, since there was no suitable sample flow for the laboratory diagnosis from the farm where there were 150 sheep and 17 aborted lambs, only biosecurity applications and medication treatment procedures were carried out in this herd. In the farm, where 700 sheep and 35 aborted lambs took place, in addition to these applications, diagnosis of the infectious agent was also carried out. All prophylactic and metaphylactic practices were carried out in the farms within the scope of routine veterinary services through the contributions of an authorized veterinary clinic in Şavşat district of Artvin province.

RESULTS

In this outbreak, the enzootic abortion cases were evaluated in two herds consisting of 850 Hemşin sheep. The herds did not have a history of vaccination against *C. abortus* infection. It was reported that there have been no feed changes and drug administrations before the outbreak. It was reported that the herds were exposed to a 2-h open-air trip in a slow rhythm at a temperature of approximately 5°C from a relatively high altitude to a low altitude area before the outbreak begins. The abortions which started the day after the trip occurred as total 52 abortions (35 abortions in 700 head sheep herd and 17 abortions in 150 head sheep herd) within 15 days, including the first abortion, diagnosis and drug administration processes. The vast majority of the abortions (45 abortions) was before the drug administration, and only 7 lamb abortions were observed in two flocks after the drug administration. The abortions were generally observed in sheep in the last 20-30 days of the gestation. It was reported that the symptoms such as loss of appetite, stillness, wet tail and genital discharges were observed just before the abortions. No other findings were observed in aborted fetuses other than generalized edema.

Cultural Analysis Findings

The common bacterial abortive agents, *Brucella* spp., *Campylobacter* spp. and *Salmonella* spp. were investigated in the fetal tissue samples by cultural methods. However, the aforementioned agents were not isolated from any of the five fetal tissues evaluated for this purpose.

Molecular Analysis Findings

Conventional PCR findings: As the results of conventional PCR analysis, the amplified products 300 bp in length specific for *C. abortus* were obtained from all of the three aborted fetal tissues *(Fig. 1)*.

Real-time PCR findings: With the real-time PCR method, two aborted fetal tissues were investigated for the viral, bacterial and parasitic abortive agents and only *C. abortus* nucleic acids were detected in the samples *(Fig. 2)*.

Immunohistochemical Analysis Findings

Hematoxylin & Eosin staining findings: In histopathological examinations, thickness in interalveolar septum was observed in some areas of lung tissue *(Fig. 3)*. Apart from these findings, no significant lesion was observed in the lung. In liver tissue, inflammatory infiltration areas consisting

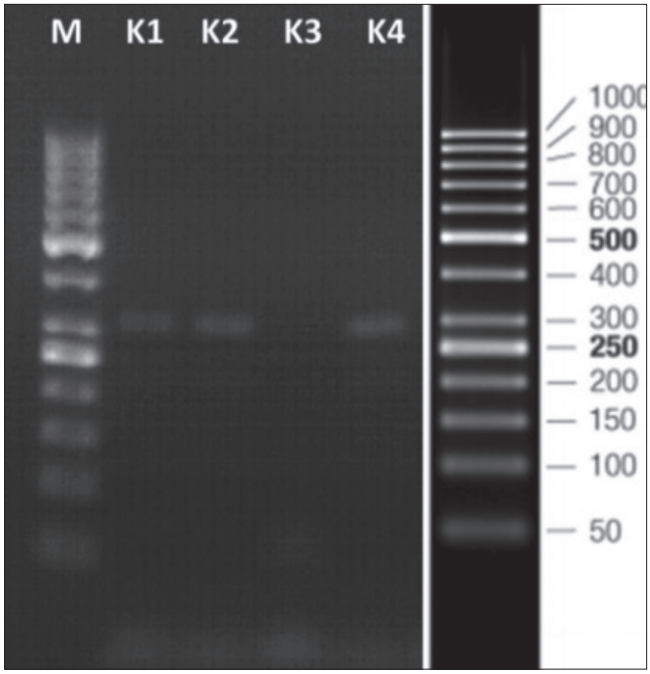


Fig 1. Electrophoresis image of conventional PCR products specific for *pmp* gene of *C. abortus*. M: Marker (Thermo Fisher Sci., SM0371), K1, K2, K4: Fetal tissue samples, K3: *E. coli* OP50

of mostly mononuclear cells and a small number of neutrophil granulocytes were detected in portal area and around vena centralis *(Fig. 4-A,B,C)*. Moreover, focal necrosis and activation in Kupffer cells were among the other important histopathological findings observed in the liver tissue.

Immunohistochemical findings: *Chlamydophila* spp. immunopositivity was detected in brown in the cytoplasm of hepatocytes *(Fig. 5)*.

Outbreak Management Findings

During and after the abortion storm, aborted sheep were identified and separated from the herds due to the risks of spread and transmission of the bacteria with genital excretes and these were housed in an isolated area. These animals were subjected to an intense care and feeding regime for 3-4 weeks until the animals recover from the postpartum period and became butchery. Moreover, these animals received special treatment to minimize the spread of the bacterial agent. In this context, the aborted sheep were treated with 5 mL/sheep I.M. of oxytetracycline (100 mg/mL) (Primavilin® Inj., Vilsan, Turkey) for 5 times 24 h

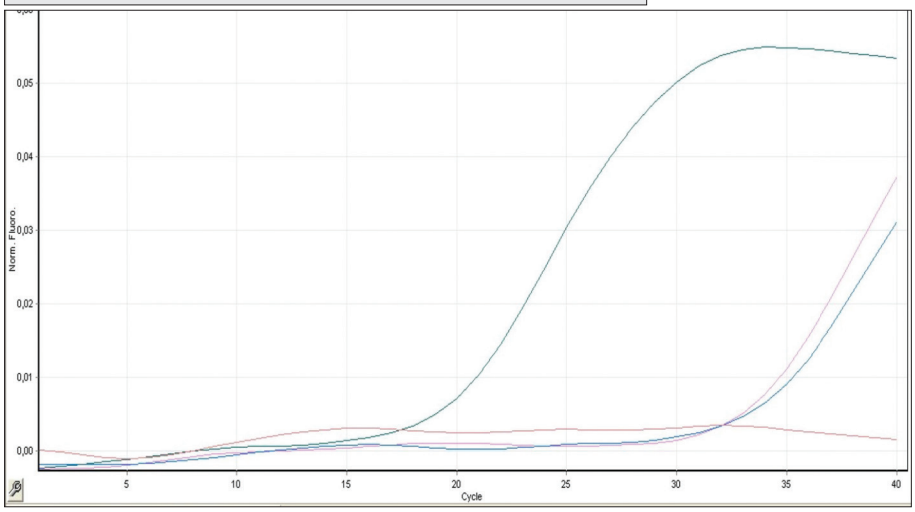
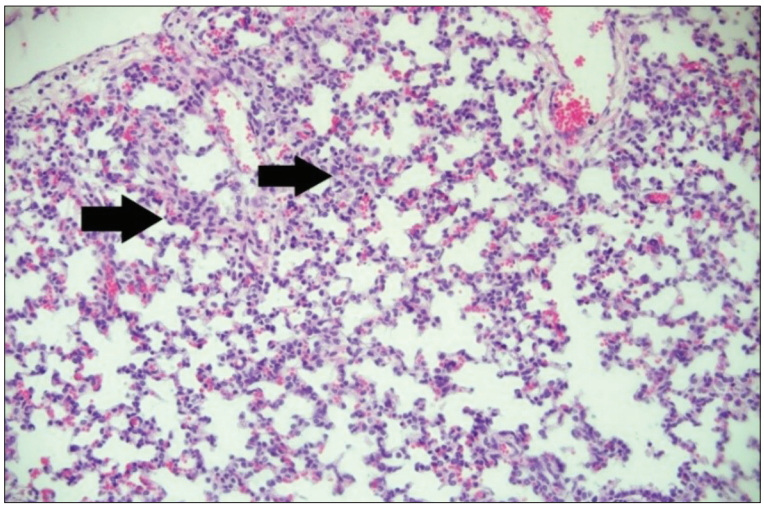


Fig 2. *C. abortus*-specific amplification curve *(pink and blue)* of real-time PCR of nucleic acid samples of two fetuses (Positive control: blue curve, Negative control: horizontal pink curve)

Fig 3. Lung tissue, thickness in the interalveolar septa *(arrows)*, H&E, Bar = 100 µm



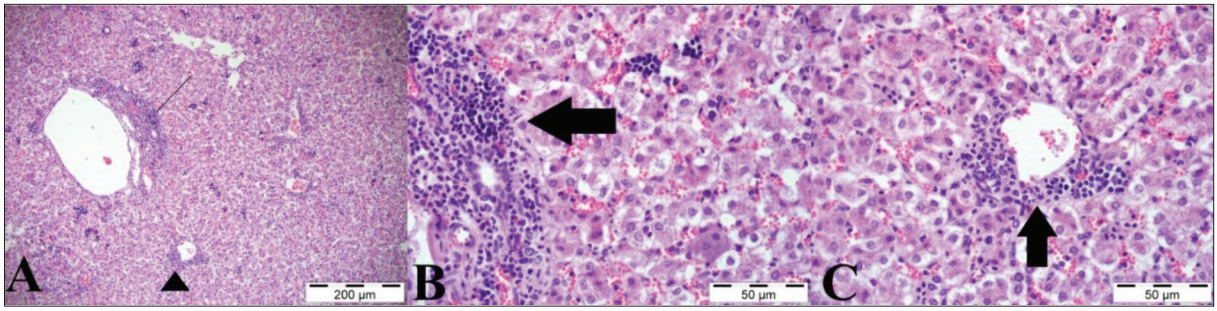


Fig 4. Liver, **A:** Cell infiltration around portal area and vena centralis, H&E, Bar = 200 µm, **B:** Higher magnification, cell infiltration around the portal area, H&E, Bar = 50 µm **C:** Higher magnification, cell infiltration around vena centralis, H&E, Bar = 50 µm

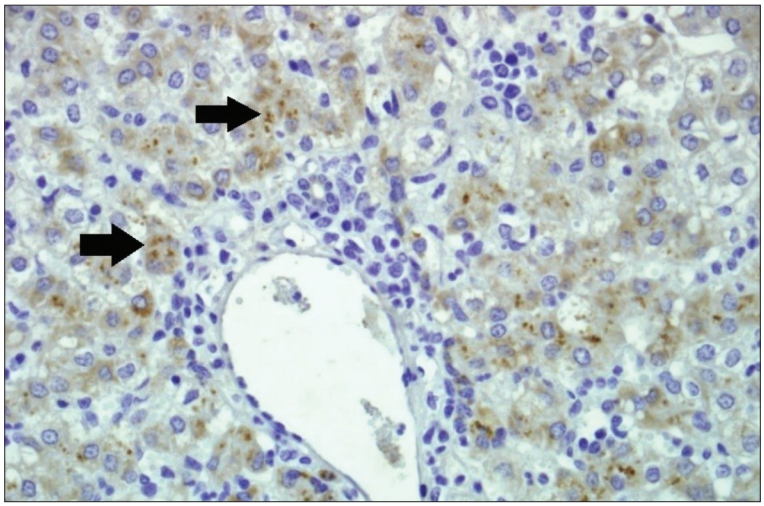


Fig 5. Liver, severe immunopositive reaction of *Chlamydophila* spp. in the cytoplasm of hepatocytes, IHC, Bar = 50 µm

apart. Additionally, the ground of the barn where the aborted sheep were sheltered was decontaminated with 0.5% of bleach once a day for 3 days.

Within the scope of biosafety practices in herds, aborted materials were covered with quicklime and buried in the soil at a depth of approximately 1.5 m and contaminated areas were disinfected with 0.5% bleach once a day for 3 days. During this period, care was taken not to allow the animals to enter and/or exit to the herds.

During the study, only one of the herds could be diagnosed with the infection. Since the story, timing and clinical reflection of the outbreak of the herd consisting of 150 sheep coincided with the other herd, a similar treatment approach was applied to this herd without a diagnosis. In this context, a common treatment regimen was applied to approximately 800 pregnant sheep in both herds to prevent abortion. For this purpose, all pregnant sheep were administered 5 mL/sheep I.M. of oxytetracycline (215 mg/mL) (Primavilin LA® Inj., Vilsan, Turkey) for 3 times as 3 days after the first application and 5 days after the second application. Moreover, a mineral-amino acid supplement (Depomin® Oral Solution, Vetaş, Turkey) was administered to the pregnant sheep by adding 15 mL of the drug to 0.5 L water for each sheep for 3 days. For prophylactic purpose, a commercial tetracycline (500 mg/g) (Tetramed® WSP, Medicavet, Turkey) with a dose of 20 mg/kg bw/day was recommended to add drinking water once a day for 3-5 days following the initial treatment.

In this study, a protective vaccine application could not be performed in the affected herds due to the lack of commercial *C. abortus* vaccine in the national market. Moreover, serological techniques in which disease was diagnosed by measuring the antibody levels to be formed approximately 2-3 weeks after the abortion were not applied on the grounds that they would not be helpful in the diagnosis of this acute course infection. The sheep herds were monitored continuously for 4-5 weeks after the outbreak, while all sheep gave healthy birth and no additional abortion, stillbirth or weak lamb births were reported. The sheep herds will continue to be monitored closely during the next few birth seasons.

DISCUSSION

"Good veterinary practices" for the correct and timely management of the outbreaks caused by the infectious agents in sheep breeding are extremely important. In this context, the biosafety practices such as diagnosis of infectious disease, isolation of infected animals and disinfection of contaminated areas, treatment of infected animals and vaccination of healthy animals, are the main

methods to be applied [10,11]. To detect infected or porter animals and determine the source of infection are the first applications to be made in an outbreak management. The main infection sources in abortive cases are placenta and vaginal discharges of the aborted sheep, and fetal membranes and tissues of the aborted fetus. Isolation and PCR methods can be used to detect *C. abortus* and the other abortive microorganisms in such samples taken freshly and maintained properly. However, *in vitro* culture of the agents with intracellular characteristics such as *C. abortus* cannot be done and thus PCR techniques are used in direct diagnosis of these agents [4,16]. Rapid and reliable diagnosis of the agent in an outbreak is very important in preventing the spread of the disease and thus in reducing economic losses. In this respect, PCR techniques have an important advantage and can identify the genus, species and even subspecies of the microorganisms [26]. The PCR techniques providing the amplification of bacterial outer membrane protein A *(ompA)* and polymorphic membrane protein *(pmp)* genes have been reported to be highly effective in identification of the *C. abortus* from clinical samples [16,19,27]. In this study, PCR techniques were applied in addition to the unsuccessful isolation attempts of the agents from the aborted materials, and direct identification of *C. abortus* from the tissue samples was performed with PCR methods. Appropriate sampling was possible from only one of the herds of sheep where the outbreak was observed and *C. abortus* positivity was achieved in 2 of these aborted lambs with real-time PCR and in 3 with conventional PCR. Tissue samples examined by the cultural methods and PCR were found negative in terms of other abortive agents. In Turkey, the most of the studies of *C. abortus* abortions are serosurvey studies showing the individual or herd-based prevalence of the agent. In these studies, the prevalence of the *C. abortus* was reported between 5.8% and 32% [28-30]. Considering this study, the average abortion rate in two herds was 6.11% (5% in the herd with 700 sheep and 11.33% in the herd with 150 sheep) and the number of abortion diagnosed as *C. abortus* was limited to 5 (9.61%) cases. Although the identification rate of this study is close to some studies, it has not been possible to compare directly with other studies in which only seroprevalence was reported [28-30]. The value of the serological diagnosis, in which a retrospective screening of the Chlamydophila infection can be made by measuring the level of antibodies formed in 2-3 weeks following the abortion, is quite limited in the period when the abortion storms are observed. Therefore, herd screening was not performed with the serological diagnostic tests on the grounds that it would not beneficial during the 15-day period when abortion storms were seen and it was postponed for use in the following season.

Histopathological and immunohistochemical diagnosis of the infection was performed on the fetal tissues of 5 aborted lambs belonging to only one enterprise. Although the findings of histopathological analysis obtained in this study are not diagnostic, they are similar to those [6,31] reported for *C. abortus* infection. Immunohistochemical (IHC) analysis is a proven technique used in confirmation of the diagnosis of *C. abortus* infection [32]. In this study, an immunopositivity was obtained in liver hepatocytes of the samples with the IHC technique using an anti-Chlamydia antibody, which recognizes the common LPS antigen of all *Chlamydia* species. Although the primary location of *C. abortus* in the abortion cases is placenta [31], in this study, IHC positivity obtained from the fetal tissues and the confirmation of the cases with PCR methods reveals the diagnostic value of these tissues in the diagnosis of infection.

Another step of the successful outbreak management is biosecurity practices, including external and internal measures to prevent transmission and spread of the infectious agent [10,11]. In this context, restriction of the animal movements and treatment of contaminated areas are the main applications. In this outbreak, besides bury of the aborted materials covered with quicklime, disinfection of contaminated enterprise areas with 0.5% bleach was also carried out for 3 days. Additionally, free movements of the herds were prevented. In enzootic abortion cases, applications related to retaining or weeding of the aborted sheep in herd are not uniform. Nevertheless, it should be considered these sheep can spread the microorganism despite being immunized following the infection. The practices related to weeding of infected animals vary depending on herd size and especially economic concerns. While it is foreseen that all animals in small herds can be disposed of following the outbreak, this practice is not very economical in large herds [10,11]. In this context, 52 sheep with abortion in these enterprises, which contain 850 sheep in total and have a relatively large herd structure, have been insulated and maintained in a separate area until the postpartum period has passed. These animals, which have been treated with special antibiotics to reduce their active shedding of the bacteria with their genital excretes, were evaluated as butchery after 3-4 weeks, paying attention to the clearance period of the administered drugs from the body. *C. abortus* poses a risk to humans due to its zoonotic feature. The transmission of the bacteria to humans is through especially contact with the infected sheep or aborted materials. *C. abortus* causes respiratory system and cardiovascular diseases in humans, as well as abortion in pregnant women [33]. In this context, in order to prevent possible human transmission and subsequent infections, biosecurity and protection measures have been described in order to fully comply with all individuals at risk in all stages of the outbreak management.

Therapeutic and curative treatment procedures in herds following the arisen of a clinical disease and the diagnosis of infection are the other useful applications in the outbreak management [10,11]. For treatment, tetracycline preparations have widely used in such applications for

Chlamydial infections in veterinary medicine [34]. Metaphylaxis also called control treatment, is the mass medication performed to prevent infection in animals at risk during an outbreak. Moreover, metaphylaxis is carried out to prevent shedding of the microorganism via the animals in incubation period or subclinically infected [35]. In this context, as soon as the infection was diagnosed in a herd, approximately 800 sheep were treated with oxytetracycline in order to prevent possible transmission and abortion in pregnant sheep. Besides, mineral-amino acid supplements were applied to the pregnant sheep. Prophylaxis called preventive treatment is a treatment approach performed in a population for preventive purposes before the infectious disease occurs [35]. In this context, there are some useful applications available regarding to add tetracycline preparations with a dose 150-500 mg/sheep/day to feeds until the end of the lambing season [5,36]. In this outbreak, it was recommended to owners to add a commercial tetracycline preparation to the drinking water once a day for 3-5 days following the preliminary antibiotic treatment. While the metaphylactic and prophylactic applications prevent the spread of Chlamydial agents and additional placental damages in body, they cannot completely eliminate the infection and reduce the existing placental damage. For this reason, new abortions and stillbirths may still be encountered after the drug administrations [5]. There is a similar situation in this outbreak, and fewer abortion cases were encountered in both herds following the drug administrations and the abortion ended within one week.

Vaccination is another method to be applied within the scope of the outbreak management in protection against the infectious diseases [10,11]. Although there are several international trademark vaccines of *C. abortus*, most of them are live vaccines whose have some restrictions such as limited uses in pregnancy and mating season, and these can not be combined with antibiotics, especially tetracyclines. In this study, it was not possible to perform a protective immunization during the outbreak in affected herds due to the absence of vaccines in the national market. Additionally, international vaccination has not been attempted to provide since the herds of late-pregnant sheep had concerns about the above vaccine restrictions. However, due to the need to continue vaccination indefinitely in reducing the incidence of infectious diseases [5], the breeders were advised to vaccinate all healthy animals against *C. abortus* after the birth season in these herds.

In conclusion, in an abandoned sheep herd, the abortion rate can reach up to 30% because of the high contagiousness of *C. abortus* and this leads to significant economic losses in sheep enterprises. The "Good Veterinary Practices" performed accurately and timely during the management of an outbreak such as biosafety measures, treatment, vaccination and close monitoring of the herd throughout this process is extremely important and can reduce the economic losses caused by the infection. In this context, it is hoped that this study, which implements the aforementioned practices that involve an accomplished management of a *C. abortion*-based outbreak in local sheep enterprises, will be beneficial for its stakeholders.

Conflict of Interest

The authors declared that there is no conflict of interest.

Acknowledgment

We would like to thank the employers for their attention, meticulous and understanding behaviours during the diagnosis, referral and management of the epidemic. We would also like to thank Veterinarian Mehmet Ali Baba for his services in prophylactic and metaphylactic practices.

Author Contributions

FB, EKÖ and EK designed the experiment, made the microbiological and histopathological analyses and wrote the manuscript. MRC, EB, MÖ and HN took over the operational stage of the epidemic management. All authors made a substantial contribution to discuss the results and approved the final version of the manuscript.

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