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Pathological, immunohistochemical and molecular studies on *Chlamydia* spp. in cattle, sheep, and goat abortions

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Abstract

This study, aimed to investigate *Chlamydia abortus*, *C. pecorum,* and *C. psittaci* in bovine, sheep, and goat abortions sent to the Konya Veterinary Control Institute diagnostic laboratory from various provinces in Central Anatolia and Mediterranean regions of Turkey between 2019 and 2022 via pathological and molecular methods. For this purpose, a total number of 150 abortions (from 58 cattle, 73 sheep, and 19 goats) were analysed via quantitative real-time polymerase chain reaction, histopathology and immunohistochemistry. The chlamydial DNA positivity rate was determined to be 24.00% via quantitative polymerase chain reaction analyses. According to the animal species, the positivity rate was 45.20% in sheep and 15.78% in goats. No cases positive for chlamydial DNA were found in the cattle abortions analyzed in the present study. When chlamydial agents were analysed on a species basis, *C. abortus* and *C. pecorum* were detected in 22.66and 1.33% of the cases, respectively. Among the animal species, 42.47% were *C. abortus* positive, whereas, 2.74% were *C. pecorum* positive in sheep. In the present study, the percentage of *C. abortus*-positive goats was 15.78%. and 22.66% of the *Chlamydia* spp. were positive according to immunohistochemical staining of aborted fetal lung, liver, heart, placenta and umbilical cord samples. This comparative study revealed that *C. abortus* was the most common chlamydial abortion agent in Türkiye. Chlamydial abortion agents cause abortion mostly in sheep in the region according to the animal species, and immunohistochemical staining contributes to the diagnosis of the agent at the *Chlamydia* spp. level.

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Introduction

The primary goal of ruminant farms is to obtain healthy offspring and high meat and milk yields, thus, increasing profitability.[1] To maintain profitability in animal husbandry, the aim is to have at least one healthy offspring per year. Abortions are among the most important factors that negatively affect this goal and cause significant economic losses in animal husbandry.[2] Abortion factors that can occur at any stage of pregnancy are classified as infectious or noninfectious causes.[3] Non-infectious causes include care and nutritional disorders, environmental conditions, and misuse of hormones and drugs.[4] Bacterial, viral, parasitic and fungal agents are among the infectious agents that play a much more significant role in the etiology of abortion. Despite the different prevalence rates among countries, the most important infectious causes of abortion in cattle, sheep and goats in Türkiye are *Brucella abortus*, *B. melitensis*, *Campylobacter fetus* subsp*. fetus*, *Chlamydia abortus*, *Salmonella abortusovis*, Akabane virus, Border disease virus, Bovine herpes virus-1, Bovine herpes virus-4, Bovine viral diarrhea virus, Bluetongue virus, *Toxoplasma gondii*, *Coxiella burnetii,* and *Neospora caninum*.[1,5]

Chlamydia, an obligate intracellular and gram-negative bacterium, is a crucial abortion agent in cattle, sheep, goats, pigs and humans. Ruminants are most commonly infected by *C. abortus* and *Chlamydia pecorum* and less frequently by *Chlamydia psittaci*. According to published studies worldwide, *C. abortus* is not the only cause of abortion in ruminant herds, but *C. pecorum* and *C. psittaci* also threaten ruminant herds.[6-9] In Türkiye, studies on *C. abortus* have emerged.[10-13]

This study aimed to investigate *C. abortus*, *C. pecorum*, and *C. psittaci* species in cattle, sheep and goat abortion that had not been treated with other abortion agents (*Brucella* spp., *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., Akabane, Border disease, Bluetongue virus, Peste des

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petits ruminants virus, *N. caninum*, *T. gondii,* etc.) according to laboratory records and submitted to Konya Veterinary Control Institute from some provinces in Central Anatolia and Mediterranean regions of Türkiye country by quantitative polymerase chain reaction (qPCR) to determine the localization of *Chlamydia* spp. in tissues and cells via immunohistochemistry (IHC) and to reveal patho-morphological changes via histopathological examinations.

Materials and Methods

Study design and sampling. When calculating the sample size of this study, the power (power of the test) for each variable was determined by taking at least 0.80 and the 1st type error 0.05. In the present study, a total number of 150 abortions (58 bovine fetuses, 73 sheep fetuses, and 19 goat fetuses) that were negative for other bacteria, viruses and parasitic abortion agents (*Brucella* spp., *Campylobacter* spp., *Salmonella* spp., *Listeria* spp., Akabane, Border disease, Bluetongue virus, Peste des petits ruminants virus, *N. caninum*, *T. gondii,* etc.) that were collected according to laboratory records. The fetuses from Afyonkarahisar 28, Aksaray 11, Antalya 16, Burdur 13, Isparta 8, Karaman 8, Konya 52, and Nigde 14 were analysed. The samples wer collected from 150 different herds. Within the scope of the study, 150 lungs, 150 livers, 150 heart tissues, 45 placentas and 59 umbilical cords were collected at the organ level. The samples were subjected to molecular analyses. Pathology revealed 114 lungs, 114 livers, 114 hearts, 21 placentas and 53 umbilical cords that were not autolyzed and suitable for examination. This study was approved by the Ethics Committee of Selcuk University, Faculty of Veterinary Medicine, Experimental Animal Production and Research Center (Approval No. 2020/44).

Nucleic acid extraction. Lung, liver, heart, placenta and umbilical cord tissues obtained after the necropsy of aborted fetuses were divided into small pieces, mixed in a biosafety cabinet and placed in a single tube for each fetal sample. The DNA extraction was performed with an IndiSpin Pathogen Kit (Indical Bioscience, Leipzig, Germany) in an automated extraction device (QIAcube; Qiagen, Hilden, Germany) according to the manufacturer's protocol. The extraction products were stored at – 20.00 ˚C until analysis.

Quantitative polymerase chain reaction (qPCR). The qPCR analysis was performed on a Qiagen Rotor-Gene Q (Qiagen). The analyses were performed according to modified company protocols using primer-probe sets targeting the *ompA* gene,[14,15] and a LightCycler 480 Probe Master Kit (Roche Diagnostics GmbH, Mannheim, Germany), (Table 1). After adding 15.00 µL of master mix, the volume was adjusted according to the number of samples studied and 5.00 µL of purified genomic DNA was added to the qPCR tubes placed on cooled 96-well plates. In addition to the samples, one positive control tube and one negative control tube were added. The tubes were placed in groups in the PCR device. A qPCR test was subsequently performed by modifying the reaction conditions reported by Pantchev *et al*.[14,15] In this context, a thermal cycling program consisting of initial PCR activation at 95.00 ˚C for 10 min, denaturation at 95.00 ˚C for 15 sec and extension at 50.00 ˚C for 2 min was applied for 40 cycles. The analysis program Rotor-Gene Q-Pure Detection Software (version 2.3.5; Qiagen, Redwood City, USA) displayed and evaluated the results.

Quantitative polymerase chain reaction (qPCR) reference controls. *Chlamydia abortus* S26/3, *C. pecorum* E58, and *C. psittaci* 6BC, positive control DNAs obtained from the Pathology Laboratory of the Faculty of Veterinary Medicine, University of Zurich, were used in the qPCR analyses. Nuclease-free water (Roche) was used as a negative control. Positive control DNAs were extracted to determine the intralaboratory reproducibility of the qPCR assays and the efficiency of the positive controls. Ten fold serial dilutions (1.00×10^0 - 1.00×10^{-7}) of positive control DNA master stocks were prepared with nuclease-free water. The qPCR assays were performed from each dilution series and cycle threshold values for positive control dilutions were determined. Accordingly, the specific amplification signal before the lowest cycle threshold value was obtained was considered positive, whereas, those after to lowest cycle threshold value were considered negative.

Histopathology. Tissue samples taken during necropsy and kept in 10.00% buffered formalin were trimmed (4.00 - 5.00 mm) for a second fixation, placed in tracing cassettes and placed in freshly prepared 10.00% buffered formalin solution for 10 - 12 hr. The fixation

Table 1. Primer-probes used in quantitative polymerase chain reaction.

Agents	Primer and prob sequences (5'–3')	Amplicon Size (bp)
Chlamydia abortus	F: 5'-GCAACTGACACTAAGTCGGCTACA-3' R: 5'-ACAAGCATGTTCAATCGATAAGAGA-3' P: FAM-AAATACCACGAATGGCAAGTTGGTTTAGCG-TAMRA	82
Chlamydia psittaci	F: 5'-CACTATGTGGGAAGGTGCTTCA-3' R: 5'-CTGCGCGGATGCTAATGG-3' P: FAM-CGCTACTTGGTGTGAC-NFQ-01-MGB	76
Chlamydia pecorum	F: 5'-CCATGTGATCCTTGCGCTACT-3' R: 5'-TGTCGAAAACATAATCTCCGTAAAAT-3' P: FAM-TGCGACGCGATTAGCTTACGCGTAG-BHQ1	76

solution was removed by washing under tap water for 24 hr. The cassettes were then passed through a series of 70.00, 80.00, 90.00, 96.00%, and absolute alcohol followed by three different xylols and then placed in a xylol-paraffin mixture. The tissues were then blocked in soft paraffin (melted at 46.00 - 48.00 ˚C) and hard paraffin (melted at 56.00 - 58.00 ˚C). These procedures were performed in an automatic tissue tracking device (Citadel 2.000; Shandon, Cheshire, UK). After the alcohol-xylol tracking process, the tissue samples were blocked with paraffin via a tissue-blocking device (Tissue-Tek TEC 5; Sakura, Torrance, USA). From the prepared paraffin tissue blocks, 4.00 - 5.00 µm thick sections were taken on slides (Marienfeld, Königshofen, Germany) with a microtome (HM 355S; Thermo Scientific, Kalamazoo, USA). The slides were dried in an oven (EN 055/120; Nüve, Ankara, Türkiye) and stained via the Hematoxylin and Eosin method,[16] in an automatic staining device (Autostainer XLST 5010; Leica, Wetzlar, Germany). After staining, the slides were covered with a coverslip (Marienfeld) with the help of Entellan (Merck, Rahway, USA) in an automatic closing device (CV5030; Leica) and examined under a binocular light microscope (BX53F; Olympus, Tokyo, Japan). The examined samples were scored to represent the whole section under the X20 objective by modifying the method reported by Meyerholz and Beck.[17] The numerical values between zero and three obtained from scoring for all samples were summed. The total result obtained was divided by the number of tissues examined and the mean severity of each finding was determined.

Immunohistochemical examination. Sections 4.00 - 5.00 µm thick were taken from the prepared paraffin tissue blocks on polylysine slides (Isotherm, Istanbul Türkiye). The sections were dried in the oven at 60.00 ˚C. Staining was then performed in the BenchMark Special Stains instrument (Ventana Medical Systems Inc., Marana, USA) following the ultraVIEW Universal Diaminobenzidin Tetrahidroklorür Detection Kit procedure (Ventana Medical Systems). For this purpose, all the preparations were labelled with barcodes containing protocol and sample information, and 30 preparations were placed in the device. The staining protocol was used and the staining process started. According to the protocol, deparaffinization was first performed with the solutions included in the kit to remove the excess paraffin in the sections. After deparaffinization, cell recovery was performed with the help of citrate buffer solutions included in the kit. A 3.00% hydrogen peroxide was applied to the preparations to reduce endogenous peroxidase activity. After this step, 125 µL of a lipo-polysaccharide-based mouse monoclonal primary antibody (Progen Biotechnik GmbH, Heidelberg, Germany) specific for the *Chlamydiaceae* family was added to each preparation at a dilution of 1/200, after which the mixture was incubated for 45 minutes. After the antibody was added, Mayer's Hematoxylin was applied to the sections, which were incubated for 8 min to allow background staining. To remove the hematoxylin residue, the sections were treated with the Bluing Reagent in the kit for 4 min. The washing processes required during the staining processes in the device were carried out with the buffer solutions included in the kit. After the staining process, the preparations taken from the device were kept in deionized water, 70.00, 80.00, 90.00, 96.00%, or an absolute alcohol series for 5 min each and in xylol for 5 min. After the preparations were ready for closure, the slides were covered with a coverslip (Marienfeld) and entellan (Merck) in the automatic closing device and examined under a binocular light microscope (BX53F; Olympus). As a result of IHC staining, granular or homogeneous brown staining with granular or homogeneous structures on a blue background was considered positive. The whole section area of the tissue was examined under 4, 10, 20, and 40 objectives. Based on Allred *et al.*,[18] modified scoring method, 12 microscopic fields representing the whole section were evaluated under an 20× objective and positive staining in each field was counted. The numerical values between zero and three obtained for all the samples were summed. The total result obtained was divided by the number of tissues examined, and the average staining intensity between 0 and 3 was determined.

Immunohistochemistry (IHC) reference controls. *Chlamydia* spp. IHC positive control preparations from the Pathology Laboratory of the Faculty of Veterinary Medicine, University of Zurich were used. Immuno-histochemical staining via sterile phosphate-buffered saline was used as a negative control instead of the primary antibody in the sections.

Statistical analysis. The categorical variables in our study are expressed as numbers and percentages. The chi-square test was used to determine the relationships between categorical variables. The z-ratio (Fisher's exact) test was used to compare the incidence rates according to subcategory. The statistical significance level was 5.00% and the Minitab (version 17.0; Minitab Inc., State College, USA) statistical package was used for calculations.

Results

Quantitative polymerase chain reaction (qPCR). A total of 24.00% (36/150) of the cattle, sheep and goats tested positive for chlamydial DNA by qPCR. Thirty-four *C. abortus* (34/36) and two *C. pecorum* (2/36) DNA samples were positive. However, no positive samples were found for *C. psittaci* DNA. According to the animal species, 45.20% (33/73) and 15.78% (3/19) of the samples were positive for chlamydial DNA from sheep and goat fetuses, respectively. No positivity for chlamydial DNA was found in fetal cattle samples (0/58). The amplification curves of some qPCR-positive samples of *C. abortus* and *C. pecorum*

are shown in Figures 1 and 2. According to the provinces for each animal species, 35.71% in Afyonkarahisar, 27.27% in Aksaray, 25.00% in Antalya, 38.46% in Burdur, 25.00% in Isparta, 21.15% in Konya and 7.14% in Nigde were positive for chlamydial DNA by qPCR. No positivity was detected in the chlamydial DNA of Karaman Province.

Gross pathology. Complete or partial autolysis was observed in the aborted fetuses of cattle, sheep and goats. In contrast, those without autolysis showed subcutaneous edema, watery or bloody contents in the abdominal and thoracic cavities and necrosis in the liver, mostly in and around the entrance of the umbilical vein but occasionally in different areas. In the placenta, necrosis was observed mainly in the cotyledons and intercotyledons.

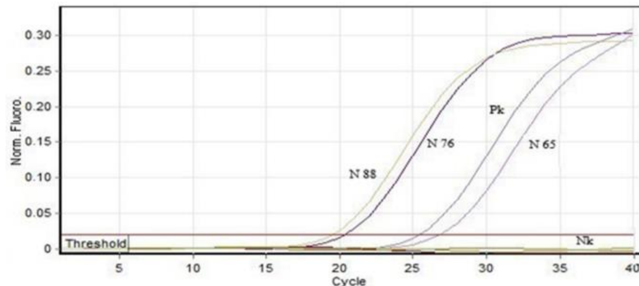

Fig. 1. Quantitative polymerase chain reaction (qPCR) amplification of several fetal samples evaluated for *Chlamydia abortus*. Pk: Positive control (1.00×10^{-3}), Nk: Negative control, N 76: Sample 76, N 65: Sample 65, and N 88: Sample 88.

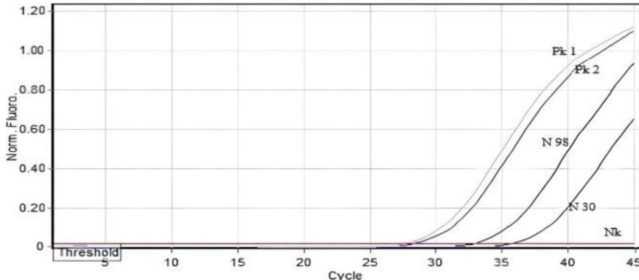

Fig. 2. Quantitative polymerase chain reaction (qPCR) amplification of fetal samples evaluated for *Chlamydia pecorum*. Pk 1: Positive control-1 (1.00×10^{-4}), Pk 2: Positive control-2 (1.00×10^{-5}), Nk: Negative control, N 30: Sample 30 and N 98: Sample 98.

Histopathology. Histopathological examination of 114 lungs, 114 livers, 114 hearts, 21 placentas and 53 umbilical cords was performed. Fetal lung samples positive for chlamydial DNA according to qPCR revealed catarrhal or mucopurulent bronchopneumonia and interstitial pneumonia of varying degrees (score = 2.16), hemorrhage (score = 2.05) and desquamation of the bronchial/ bronchiolar epithelium (score = 1.72; Fig. 3A). Fetal lung samples negative for chlamydial DNA according to qPCR revealed catarrhal or interstitial pneumonia with hyperemia (score = 1.33), hemorrhage (score = 0.53) and desquamation of the bronchial/bronchiolar epithelium (score = 1.44) of varying severity. Hydropic degeneration (score = 1.53) and necrosis (score = 1.30), hyperemia (score = 1.91), hemorrhage (score = 1.80), and neutrophil, granulocyte and mononuclear cell infiltration (score = 1.61) in the portal spaces (Fig. 3B) were detected in the fetal liver samples positive for chlamydial DNA via qPCR. Fetal liver samples negative for chlamydial DNA by qPCR revealed hepatitis of varying severity including hydropic degeneration (score = 1.67) and necrosis (score = 1.20) of hepatocytes, hyperemia (score = 0.78), hemorrhage (score = 0.95) and mononuclear cell infiltration in the portal spaces (score = 0.09). Fetal heart samples positive for chlamydial DNA by qPCR revealed myocarditis of varying severity, including degeneration and necrosis of heart muscle cells (score = 1.58), hyperemia (score = 1.61), hemorrhage (score = 1.11) and mononuclear cell infiltration in interstitial areas (score: 0.78; Fig. 3C). Similarly, in fetal heart samples that were negative for chlamydial DNA according to qPCR, varying degrees of severity were observed including degeneration and necrosis of heart muscle cells (score = 1.01), hyperaemia (score = 0.36), haemorrhage (score = 0.04) and mononuclear cell infiltration in interstitial areas (score = 0.05). In the fetal placenta samples positive for chlamydial DNA according to qPCR, acute placentitis with necrosis (score = 2.87), hyperaemia (score =1.50), oedema (score = 0.75) and haemorrhage (score = 1. 25), usually dominated by neutrophil granulocytes (score = 2.12; Fig. 3D), and subchronic or chronic placentitis, in which mononuclear cells (Score = 2.12) were more dominant in some sections. Placentitis including necrosis (score = 1.69), hyperemia (score = 1.00), edema (score = 1.69), hemorrhage (score = 1.15), and cellular infiltration, was observed in fetal

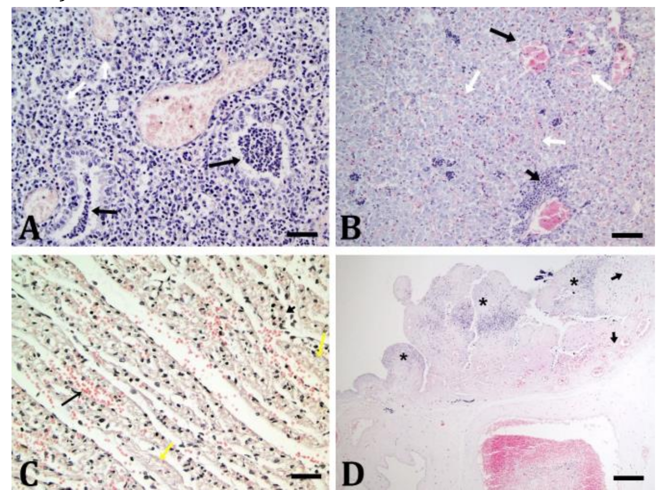

Fig. 3. A) Lung. Exudate (black arrows) in bronchiolar lumens and macrophages (white arrows) in alveoli. **B)** Liver. Hyperemia in the central vein (long black arrow) and sinusoids (white arrows), mononuclear cell infiltration in the portal area (short black arrow), **C)** Heart. Hyperemia (long black arrow), degeneration (yellow arrows), and mononuclear cell infiltration (short black arrow), and **D)** Placenta. Necrosis (astrickes), hyperemia (black arrows), (Hematoxylin and Eosin staining; bars = 400 µm).

placenta samples, which were negative for chlamydial DNA by qPCR. Fetal umbilical cord samples positive for chlamydial DNA according to qPCR showed omphalitis including necrosis (score=1.25), hyperemia (score = 1.60), edema (score = 0.88), hemorrhage (score = 1.06) and inflammatory cell infiltration (score = 1.31) of varying severity. Fetal umbilical cord samples that were negative for chlamydial DNA according to qPCR revealed omphalitis which included necrosis (score = 1.08), hyperemia (score = 0.89), edema (score = 1.27), hemorrhage (score = 0.67) and cell infiltration (score = 0.08) of varying severity.

Immunohistochemical (IHC). The IHC was performed on all tissues suitable for histopathological examination. The IHC positivity rate for *Chlamydia* spp. was 22.66% (34/150). All samples that were positive by IHC were also positive according to qPCR. For two samples with positive qPCR results, positivity was not detected via IHC. IHC revealed no immunopositivity in any of the samples negative for chlamydial DNA. Positive staining was detected in 22.80% (26/114), 26.31% (30/114), 28.07% (32/114), 38.09% (8/21) and 24.52% (13/53) of the lung, liver, heart, placenta and umbilical cord, respectively. As a result of the scoring, the mean staining intensity in the range of 0-3 according to the tissues was 1.09 in the lung, 1.35 in the liver, 1.38 in the heart, 2.50 in the placenta, and 1.25 in the umbilical cord. The IHC revealed that chlamydial antigens were more highly localized in trophoblasts in the placenta (Fig. 4D), alveolar and peribronchiolar macrophages in the lung (Fig. 4A), inflammatory infiltration in the portal area in the liver (Fig. 4B), inflammatory infiltration in the interstitial area in the heart (Fig. 4C) and in the amnion epithelium and subepithelial areas in the umbilical cord, and that the staining was granular or homogenous.

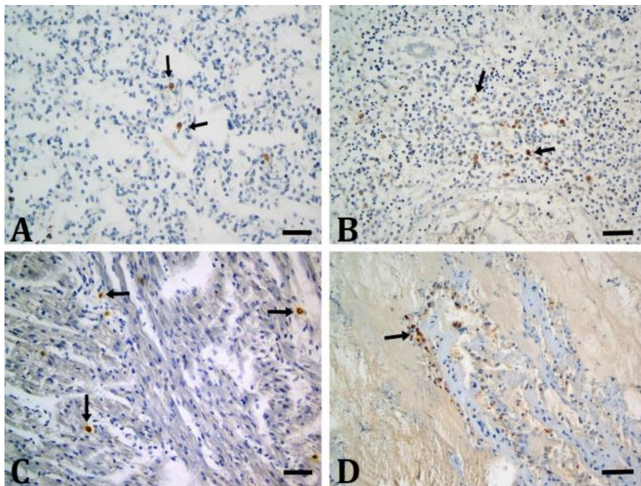

Fig. 4. Positive immunostaining of *Chlamydia* spp. **A)** Alveolar macrophages of the lung. The arrows in the image indicate *Chlamydia* spp. positive IHC labelling within alveolar macrophages in the lung, **B)** Inflammatory cells in the portal region of the liver. The arrows in the image indicate *Chlamydia* spp. positive immunohistochemistry (IHC) labelling in inflammatory cells in the portal area of the liver, **C)** Interstitium of the myocardium. The arrows in the image indicate *Chlamydia* spp. positive immunohistochemical labelling in the myocardium's interstitium, and **D)** Trophoblast cells in the placenta (arrows), (IHC staining; bars = 400 μm).

Statistical results. According to the distribution of *Chlamydia* spp. positivity according to province and proportional comparison results, the difference in *Chlamydia* spp. positivity rates between provinces was not statistically significant ($p > 0.05$). In other words, abortion-related factors were not significantly different among these provinces. When analysed on the basis of province, the *Chlamydia* spp. positivity rate (4/16) in Antalya Province was statistically significant ($p = 0.027$). Accordingly, *Chlamydia* spp. were present at a significant level in this province. In Konya Province, the *Chlamydia* spp. positivity rate (11/52) was significantly different ($p = 0.001$). *Chlamydia* spp. is important in this province. Similarly, in the percentage of *Chlamydia* spp. positive individuals (1/14) in Nigde Province was significantly different ($p = 0.002$). Accordingly, *Chlamydia* spp. were present at a significant level in this province. Finally, there was no *Chlamydia* spp. positivity in Karaman Province and this difference (0/8) was statistically significant ($p = 0.008$). On the other hand, for *Chlamydia* spp. the positivity rate was not significantly different in provinces other than those mentioned above ($p > 0.05$). When the distribution of *Chlamydia* spp. positive IHC staining according to organ and the results of proportional comparisons were analysed, the difference in IHC-positive staining rates between organs in terms of *Chlamydia* spp. was not statistically significant ($p > 0.05$). In other words, the difference in the percentage of IHC-positive cells was insignificant between these organs. When analysed on an organ basis, the percentages of IHC-positive *Chlamydia* spp. cells in the lung, liver, heart and umbilical cord were significantly different ($p < 0.05$). However, the percentage of IHC-positive *Chlamydia* spp. staining in the placenta was not significantly different ($p > 0.05$).

Discussion

An examination of related studies revealed that *C. abortus* and *C. pecorum* are essential causes of abortion in domestic ruminants.[19,20] In recent studies, *C. psittaci* has also been reported to cause abortion in ruminants.[20,21] In this study, aborted fetal materials from provinces in the region responsible for the Konya Veterinary Control Institute, including Konya Province, were studied. In terms of chlamydial DNA, 38.46% ($p = 0.581$) were from Burdur, 35.71% ($p = 0.185$) were from Afyonkarahisar, 27.27% ($p = 0.227$) were from Aksaray, 25.00% ($p = 0.227$) were from Antalya, 25.00% ($p = 0.027$) were from Isparta, 25.00% ($p = 0.289$) were from Konya, 21.15% ($p = 0.001$) and 7.14% were from Nigde ($p = 0.002$), whereas, no

positive cases were found in Karaman (p = 0.008). In contrast, *C. pecorum* was detected in only one case each in Burdur and Afyonkarahisar and *C. abortus* DNA was detected in the materials from all provinces except for KaramanApart from serological study results concerning *chlamydia*-related abortions in the region, there are very few molecular-based studies. In serological studies, Öztürk *et al.*,[22] reported *C. abortus* seropositivity in 32.00% of sheep blood sera in Burdur, and Kaya and Öztürk,[23] reported *C. abortus* sero-positivity in 19.27% of goats in Burdur. In the present study, the prevalance of chlamydial DNA positivity was 38.46% in Burdur and 21.15% in Konya. When the data were compared, they were concordant, and it was predicted that chlamydial agents may be an essential problem for these provinces. Chlamydial DNA positivity was not detected in Karaman Province because of the low number of fetal samples examined.

In previous studies conducted in Türkiye on chlamydial abortions via classical PCR, Güler *et al.*,[24] reported 7.50% of sheep vaginal swab samples from the Konya region, Kılıç *et al.*,[25] reported 6.00% of bovine fetus samples from Elazıg Province, Kalender *et al.*[26] reported 9.80%; 14.29% of sheep and goats aborted fetus samples, and Aras *et al.*,[27] reported 9.80and 14.29% of bovine fetus samples from the Aksaray and Konya regions, respectively. Kalender *et al.*,[26] reported 9.80 and 14.29% of sheep and goats aborted fetus samples in the Elazıg region; Aras *et al.*,[27] reported 3.00% of bovine fetus stomach content samples in the Aksaray and Konya regions; Filikci[9] reported 54.00% of goats and sheep aborted fetus/mortal birth materials; Akpınar *et al.*,[12] detected *C. abortus* positivity in 16.50% of sheep fetuses in the Black Sea region in a molecular study. Furthermore, Kanat[28] reported 15.40% *C. abortus* in aborted sheep fetuses in Konya Province. Notably, except for one of the studies mentioned above,[9] only *C. abortus* was reported. In this study, when bovine fetal samples were analysed for *C. abortus*, no positive results were obtained, unlike the findings of Kılıç *et al.*,[25] and Aras *et al.*[27] The reason for this difference was the variability, such as the periods in which the studies were carried out, regional differences and differences in the study material. Unlike in these studies, bovine fetal samples from *C. pecorum* and *C. psittaci* were negative.

When qPCR studies conducted in Türkiye in recent years were analysed, Malal and Turkyılmaz,[10] similar to our study, reported 3.00% positivity in cattle, 16.60% positivity in sheep, and 21.40% positivity in goats in their study on *C. abortus* only in different animal species. Yeni,[11] on the other hand, detected 1.30% *C. abortus* positivity in bovine-aborted fetus samples and did not report any positive cases in sheep-aborted fetus samples. When the study results were compared to these data, Malal and Turkyılmaz[10] reported a higher positivity rate in sheep and a lower positivity rate in goats, whereas, Yeni reported a very high level in sheep.[11] According to the results of the study by Yeni, a much lower number of samples were taken from sheep materials than from the samples in this study and these samples were limited to stomach contents.[11] In addition, the number of bovine aborted bovine fetuses was much greater in the study by Yeni than in the present study.[11] This finding indicated that additional material was needed to determine the causative agent in aborted bovine fetuses. Compared to the studies by Malal and Turkyılmaz[10] and Yeni,[11] the presence of *C. pecorum* in sheep and goat samples was also demonstrated in this study.

Chlamydia psittaci DNA was not detected in any of the samples analysed in this study by qPCR. *C. psittaci* abortion cases have been reported in studies conducted in different parts of the world. Borel *et al.*[19] and Tavares Clemente *et al.*,[20] reported positivity rates of 9.30% in cattle and 3.03% in goats in cattle, sheep and goat abortion samples in Portugal. Apart from the results of the present study, in Türkiye, Sakmanoğlu *et al.*[29] reported that *C. psittaci* was not found in a multiplex PCR study performed on samples taken from the stomach contents of aborted fetuses of cattle, sheep and goats sent to the laboratory from 7 different geographical regions of Türkiye for one year. According to the literature, no other data were found on cattle, sheep or goat abortions in Türkiye. There are insufficient studies on *C. psittaci* abortion in cattle, sheep and goats in Türkiye. In this context, studies with larger budgets and at the national level are needed to reveal the effectiveness of *C. psittaci* in abortion cases in Türkiye.

Chlamydia pecorum is reported to be an essential abortion agent together with *C. abortus*, especially in sheep.[30] There are many studies on *C. pecorum* in cattle, sheep and goats worldwide. In Portugal,[20] 4.65% of sheep were infected, 3.03% were infected goats and in Australia,[8] 2.73% were infected with *C. pecorum*. In Türkiye, only one study was found in the literature. This study reported a percentage of *C. pecorum*-positivity of 6.00% by the classical PCR method in aborted or stillborn sheep and goat fetuses brought to Ankara University, Faculty of Veterinary Medicine Pathology Laboratory.[9] In this study, *C. pecorum* was detected at a rate of 2.74% in the samples collected. This rate was lower than the rate given by Filikci.[9] This difference might be attributed to differences in material supply or region. The two cases of *C. pecorum* found to be qPCR positive were sheep fetuses and these samples belonged to Burdur and Afyonkarahisar Provinces. The literature searches revealed no studies on *C. pecorum* in these provinces. For this reason, the data obtained in this study were recorded first for these provinces. Considering both the data reported by Filikci[9] and the results of this study, *C. pecorum* emerged as an essential abortion agent after *C. abortus* in chlamydial abortion cases, and additional detailed studies are needed in Türkiye.

This study involved histopathological examinations of lung, liver, heart, placenta, and umbilical cord samples from aborted fetuses which were positive for chlamydial DNA according to qPCR. These findings were similar to those in the literature. In these studies, Buxton *et al.*,[31] reported suppurative placentitis, hepatitis and severe neutrophil granulocyte exudation and lymphocyte infiltration in the bronchioles of the lungs of sheep and Szeredi and Bacsadi[32] reported histopathological inflammation, necrosis and vasculitis in the placenta of *C. abortus* positive sheep and goats. Maley *et al.*,[33] reported necrosis, tissue loss, thrombosis in fetal blood vessels and neutrophil granulocyte and mononuclear cell infiltration in tissues associated with the placenta via histopathological evaluations of placenta taken from sheep during different periods of pregnancy. Borel *et al.*,[19] detected necrosis in trophoblasts and neutrophil granulocyte exudation in the placenta via histopathological examinations performed on chlamydial DNA-positive bovine tissues. In their experimental infection, Longbottom *et al.*,[34] histo-pathologically reported suppurative placentitis, vasculitis and neutrophil granulocyte exudation in the chorionic epithelium in the placenta, periportal hepatitis and necrosis in the liver, neutrophil granulocyte exudation in the alveoli, interstitial pneumonia, interlobular edema and hemorrhage in some cases in the lung. Westermann *et al.*,[8] reported necro suppurative placentitis and vasculitis in the placenta and neutrophil granulocyte and macrophage infiltration in the bronchiole lumen in the lungs in their study of sheep abortions. Finally, in their experimental study, Livingstone *et al.*,[35] histopathologically detected varying degrees of necrotizing-suppurative placentitis in infected placentas, neutrophil granulocytes; lymphocyte and macrophage infiltration in the cotyledons and inter-cotyledonary region, hemorrhage in the inter-cotyledonary region and vasculitis with vessels. In field studies conducted in Türkiye, hyperemia and mononuclear cell infiltration in various organs have been reported, although not precisely.[9] When the studies conducted in Türkiye were analysed, it was found that placental lesions were more prominent similar to the findings of this study.

According to the IHC results of fetal tissues obtained from cattle, sheep and goats, placental samples are important for the diagnosis of chlamydial agents by IHC. Chlamydial antigens have been reported to localize to structures and inflammatory infiltration areas in various tissues.[11,30,33,34,36,37] According to the IHC results obtained in this study, chlamydial antigens were more highly localized in trophoblasts in the placenta and alveolar and peribronchiolar macrophages in the lung. Inflammatory infiltration was found in the portal area of the liver, inflammatory infiltration was found in the interstitial area of the heart and the amniotic epithelium and subepithelial areas of the umbilical cord and the staining was granular or homogenous. The findings of this study indicated that in cases of abortion due to chlamydial agents, the fetus was often infected in addition to the umbilical cord and placenta. Borel *et al.*,[19] in their study on Chlamydial abortions in cattle in Switzerland, reported positivity by PCR for *C. abortus* and *C. psittaci* in placenta samples and reported positivity by IHC in all of the samples. Navarro *et al.*,[38] reported similar results in the detection of chlamydial abortion agents in placental samples via PCR and IHC in small ruminant placentas. In this study, similar to Borel *et al.*,[19] and Navarro *et al.*,[38] all samples that were positive by IHC were also positive by qPCR. In two samples with positive results by qPCR, positivity was not observed by IHC.

In conclusion, this study revealed that *C. abortus* had a significant prevalence in sheep abortions occurring in various provinces in Central Anatolia and Mediterranean regions of Turkey and that *C. pecorum* might be an important abortion agent after *C. abortus* in chlamydial abortion cases. In addition to the chlamydial abortion agents identified in this study, more detailed and comprehensive studies will be needed for other chlamydial abortion agents that cause fewer abortions. In addition, this study demonstrated that IHC could be used as an alternative diagnostic method to PCR in the diagnosis of chlamydial abortions.

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Conflict of interest

The authors declare no conflicts of interest.

References

1. Doğan F, Dağalp SB. The etiyopatogenesis of viral abortion cases in cattle [Turkish]. MAKÜ Sag Bil Enst Derg 2017; 5(1): 66-77.
2. Wu Z, Sippy R, Sahin O, et al. Genetic diversity and antimicrobial susceptibility of *Campylobacter jejuni* isolates associated with sheep abortion in the United States and Great Britain. J Clin Microbiol 2014; 52(6): 1853-1861.
3. van den Brom R, Lievaart-Peterson K, Luttikholt S, et al. Abortion in small ruminants in the Netherlands

between 2006 and 2011. Tijdschr Diergeneeskd 2012; 137(7): 450-457.
4. Ay SS, Gürler H, Önyay F, et al. Abortion problems and reproductive vaccination programs in small ruminants [Turkish]. Turkiye Klinikleri J Vet Sci Obstet Gynecol-Special Topics 2017; 3(2): 129-136.
5. Gülaydın Ö, Öztürk C, Ekin İH, et al. Investigation of selected bacterial agents causing sheep abortion in the Van Province by RT-PCR and histopathological methods. Acta Vet Brno 2023; 92(1): 69-77.
6. Clune T, Besier S, Hair S, et al. Chlamydia pecorum detection in aborted and stillborn lambs from Western Australia. Vet Res 2021; 52(1): 84. doi: 10.1186/s13567-021-00950-w.
7. Struthers JD, Lim A, Ferguson S, et al. Meningo-encephalitis, vasculitis, and abortions caused by *Chlamydia* pecorum in a herd of cattle. Vet Pathol 2021; 58(3): 549-557.
8. Westermann T, Jenkins C, Onizawa E, et al. *Chlamydia pecorum*- associated sporadic ovine abortion. Vet Pathol 2021; 58(1): 114-122.
9. Filikci K. Pathomorphology of chlamydial abortions in sheep and goats. Ph.D. Thesis, Ankara University. Ankara, Turkey: 2022.
10. Malal ME, Turkyilmaz S. Identification and genotyping of *Chlamydia* abortus with MLVA from ruminant abortions in the Marmara region of Turkey. Thai J Vet Med 2021; 51(1): 169-175.
11. Yeni DK. Molecular diagnosis of neglected infectious agents of heep and cattle abortions: the prevalences of *Coxiella* burnetii, *Francisella tularensis*, and *Chlamydophila abortus* at a glance. Ankara Univ Vet Fak Derg 2022; 69(4): 425-430.
12. Akpınar RK, Kılıçoğlu Y, Şahin B, et al. Molecular detection of *Chlamydia abortus* and investigation of oxidative stress parameters in sheep fetuses in the Black Sea Region of Türkiye. Comp Clin Pathol 2024; 33: 47-54.
13. Yeni DK, Balevi A, Ashraf A, et al. Molecular detection of bacterial zoonotic abortive agents from ruminants in Turkey. Braz J Microbiol 2024; 55(2): 1997-2004.
14. Pantchev A, Sting R, Bauerfeind R, et al. New real-time PCR tests for species-specific detection of *Chlamydophila psittaci* and *Chlamydophila abortus* from tissue samples. Vet J 2009; 181(2): 145-150.
15. Pantchev A, Sting R, Bauerfeind R, et al. Detection of all *Chlamydophila* and *Chlamydia* spp. of veterinary interest using species-specific real-time PCR assays. Comp Immunol Microbiol Infect Dis 2010; 33(6): 473-484.
16. Luna L. Routine Harris Hematoxylin and Eosin stain. New York, USA: McGraw-Hill 1968; 38.
17. Meyerholz DK, Tintle NL, Beck AP. Common pitfalls in analysis of tissue scores. Vet Pathol 2019; 56(1): 39-42.
18. Allred DC, Harvey JM, Berardo M, et al. Prognostic and predictive factors in breast cancer by immuno-histochemical analysis. Mod Pathol 1998; 11(2): 155-168.
19. Borel N, Thoma R, Spaeni P, et al. Chlamydia-related abortions in cattle from Graubunden, Switzerland. Vet Pathol 2006; 43(5): 702-708.
20. Tavares Clemente ML, Bragança Barahona MJ, Capela Andrade MF, et al. Diagnosis by PCR-REA of *Chlamydophila* species infections in late-term abortions of domestic ruminants. Vet Rec 2011; 168(23): 619. doi: 10.1136/vr.d889.
21. Doosti A, Arshi A. Molecular study for detection of *Chlamydia psittaci* caused abortion in Iranian cattle. JPAM 2012; 6(3): 1133-1138.
22. Öztürk D, Türütoğlu H, Kaya M. Seroprevalence of *Chlamydophila abortus* infection in sheep in Burdur province [Turkish]. Vet J MAKÜ 2016; 1(2): 17-20.
23. Kaya M, Öztürk D. Seroprevalence of *Chlamydophila abortus* ınfections in goats in Burdur Province [Turkish]. MAKÜ Sag Bil Enst Derg 2020; 8(1): 1-10.
24. Güler L, Hadimli HH, Erganiş O, et al. Field evaluation of a PCR for the diagnosis of chlamydial abortion in sheep. Vet Rec 2006; 159(22): 742-745.
25. Kılıç A, Kalender H, Muz A. Detection of *Chlamydophila abortus* in aborted bovine fetuses by microbiological culture and PCR [Turkish]. Fırat Üniv Sağlık Bilimleri Vet Derg 2010; 24(3): 129-132.
26. Kalender H, Kiliç A, Eröksüz H, et al. Identification of *Chlamydophila abortus* infection in aborting ewes and goats in Eastern Turkey. Rev Med Vet 2013; 164(6): 295-301.
27. Aras Z, Gökçenur SG, Sayin Z. Investigation of the presence of *Chlamydophila abortus* in bovine abortions by PCR [Turkish]. Eurasian J Vet Sci 2017; 33(2): 77-80.
28. Kanat Ö. Molecular and histopathologic investigation of Pestivirus, *Chlamydophila abortus*, and *Listeria monocytogenes* infections in aborted sheep fetuses. J Hellenic Vet Med Soc 2022; 73(1): 3889-3896.
29. Sakmanoğlu A, Uslu A, Sayın Z, et al. A one-year descriptive epidemiology of zoonotic abortifacient pathogen bacteria in farm animals in Turkey. Comp Immunol Microbiol Infect Dis 2021; 77: 101665. doi: 10.1016/j.cimid.2021.101665.
30. Quinn, P, Markey B, Leonard F, et al. Veterinary Microbiology and Microbial Disease. 2nd ed. Chichester, United Kingdom: Wiley-Blackwell 2011; 384-393.
31. Buxton D, Anderson IE, Longbottom D, et al. Ovine chlamydial abortion: characterization of the inflammatory immune response in placental tissues. J Comp Pathol 2002; 127(2-3): 133-141.
32. Szeredi L, Bacsadi A. 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 2002; 127(4): 257-263.
33. Maley SW, Livingstone M, Rodger SM, et al. Identification of *Chlamydophila abortus* and the development of lesions in placental tissues of experimentally infected sheep. Vet Microbiol 2009; 135(1-2): 122-127.
34. Longbottom D, Livingstone M, Maley S, et al. Intranasal infection with *Chlamydia abortus* induces dose-dependent latency and abortion in sheep. PLoS One 2013; 8(2): e57950. doi: 10.1371/journal.pone.0057950.
35. Livingstone M, Wheelhouse N, Ensor H, et al. Pathogenic outcome following experimental infection of sheep with *Chlamydia abortus* variant strains LLG and POS. PLoS One 2017; 12(5): e0177653. doi: 10.1371/journal.pone.0177653.
36. Karagül MS, Malal ME, Akar K. Investigation of *Coxiella burnetii* and *Chlamydia abortus* antibodies in sheep in Düzce region [Turkish]. DU Health Sci Inst J 2019; 9(3): 106-109.
37. Kreizinger Z, Szeredi L, Bacsadi Á, et al. Occurrence of *Coxiella burnetii* and Chlamydiales species in abortions of domestic ruminants and in wild ruminants in Hungary, Central Europe. J Vet Diagn Invest 2015; 27(2): 206-210.
38. Navarro JA, Ortega N, Buendia AJ, et al. Diagnosis of placental pathogens in small ruminants by immuno-histochemistry and PCR on paraffin-embedded samples. Vet Rec 2009; 165(6): 175-178.