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Original article

Investigation of the presence of *Chlamydia* spp., *Mycoplasma* spp. and *Moraxella ovis* in infectious keratoconjunctivitis cases in sheep and goats in Siirt province and evaluation of clinical findings

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Abstract

Infectious keratoconjunctivitis is an infectious disease that negatively affects animal welfare causing systemic or local clinical signs in small ruminants and causes significant economic losses in herds. It is important to determine the etiologic agent causing the infection in the development of the protection and control strategies against the disease. The aim of this study was to determine the presence of infectious keratoconjunctivitis cases in small ruminants raised in Siirt province in Türkiye. Infectious keratoconjunctivitis was graded according to the symptoms determined by clinical examination. The presence of *Chlamydia* spp., *Mycoplasma* spp. and *Moraxella ovis* was investigated by PCR in swab samples obtained from the animals with keratoconjunctivitis. Infectious keratoconjunctivitis was detected in 263 (19.86%) of 1324 animals examined in the study. Of the animals with infectious keratoconjunctivitis, 163 (61.97%) were sheep and 100 (38.02%) were goats. The detection rate of infectious keratoconjunctivitis was higher in sheep than goats. In 56 (21.29%), 109 (41.44%), 67 (25.47%), and 31 (11.78%) of the cases, findings of stage 1, 2, 3, and 4 infectious keratoconjunctivitis were detected, respectively. Of the eye swab samples taken from 263 animals with infectious keratoconjunctivitis, 5 (1.90%) were positive for *Mycoplasma* spp. and 6 (2.28%) were positive for *M. ovis*. It was determined that the distribution of the bacterial agents varied according to the stage of infectious keratoconjunctivitis. No statistically significant correlation was found in the distribution of bacterial agents among identified samples according to species, sex, age, and infectious keratoconjunctivitis stage of the animals. It was thought that the data obtained in the study would contribute to the studies for protection and control by determining the incidence and aetiology of infectious keratoconjunctivitis cases observed in small ruminants.

Keywords: *Chlamydia* spp., goat, keratoconjunctivitis, *Mycoplasma* spp., *Moraxella ovis*, sheep



Introduction

Infectious keratoconjunctivitis (IKC) is an infectious disease having symptoms such as conjunctival hyperaemia, serous or purulent discharge, blepharospasm and temporary or permanent blindness (Giacometti et al. 1999, Akerstedt and Hofshagen 2004, Işık et al. 2018). Infection affects animal welfare adversely and causes significant economic losses in small ruminant farming, weight and yield loss, decreased twin pregnancy rate, and pregnancy toxemia (Akın and Samsar 2005, Hosie 2007, Townsend 2008, Işık et al. 2018). Although IKC was first identified in 1916 in wild goats in the Austrian Alps, it has been reported in many countries until today (Romano et al. 2000). Bacterial agents such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Moraxella ovis* (*M. ovis*), *Chlamydia* spp. and *Mycoplasma* spp. play a role in the development of the disease (Naglic et al. 2000, Akerstedt and Hofshagen 2004, Fernández-Aguilar et al. 2017, Karthik et al. 2017, Işık et al. 2018).

When the clinical findings of IKC are evaluated, symptoms such as serous tear discharge in one and/or both eyes, conjunctival inflammation, cerebriform scleral vascularization, hyperaemia, blepharospasm, and photophobia are generally observed in sick animals. However, it is also highlighted that clinical symptoms may vary according to the agent isolated from the cases. Thus, keratitis and ulceration, neovascularization, and rarely descemetocoele and iris prolapse, may occur in IKC cases caused by *Mycoplasma* spp. isolates (Aguilar et al. 2013). In the infections caused by *Chlamydia* spp. isolates, epiphora, chemosis, mucopurulent tear discharge in advanced cases, and corneal ulceration are reported (Giacometti et al. 2002, Aguilar et al. 2013).

Sheep and goats may show different clinical findings in relation to the disease. While mucopurulent conjunctivitis, follicular conjunctivitis, iritis with hypopyon and corneal ulceration, and rarely phthisis bulbi are observed in advanced cases in sheep, it is determined that permanent corneal opacities and blindness occur in goats (Loison et al. 1996). Untreated cases result in mucopurulent conjunctivitis and corneal ulceration resulting in corneal opacification and temporary blindness. This blindness leads to loss of productivity as a result of inadequate nutrition and reduced economic return in sheep-goat breeding (Gelormini et al. 2017, Işık et al. 2018). It is reported that, as a result of IKC cases, mortality rates can increase to 30% in herds (Fernández-Aguilar et al. 2013, Gelormini et al. 2017).

Although the infection is estimated to be seen in the form of sporadic cases in Türkiye, there is a limited

number of related studies (Çakır et al. 2014, Gülmez Sağlam 2018). The aim of the current study was to determine the prevalence and clinical findings of infectious keratoconjunctivitis cases in sheep and goats in Siirt province and its surroundings and to identify the presence of *Chlamydia* spp., *Mycoplasma* spp. and *M. ovis* agents in the cases.

Materials and Methods

Material

A total of 1324 small ruminants (632 sheep, 692 goats) of different breeds, ages and sexes, which were raised in 13 enterprises in the city centre of Siirt province and its Tillo, Şirvan, Kurtalan, and Eruh districts between April 2021 and March 2023, were screened for IKC.

Ethics committee approval

The study was approved with the decision (dated 23.09.2020 and numbered 2020/03-05) of Siirt University Experimental Animals Local Ethics Committee.

Method

Clinical examination

Animals diagnosed with IKC were classified according to the 4 clinical stages of the disease as determined by Mayer et al. (1997). Symptoms of conjunctival hyperaemia, serous lacrimation, excessive blinking, and blepharospasm, congestion in the cornea-sclera, vascularization (pannus) towards the cornea were then classified as stage 1; corneal inflammation, extensive vascularization in the cornea, increased irritation in the eye as a result of keratitis, lacrimation and significant blepharospasm were classified as stage 2. More corneal vascularization together with mucopurulent keratitis, more purulent tear discharge, superficial corneal ulcers, and visual loss were classified as stage 3, and enlargement of corneal ulcers, pus accumulation (hypopyon) in the anterior chamber of the eye and visual loss were classified as stage 4.

Microbiological analysis

In the study, swab samples were taken from the affected eye(s) of animals diagnosed with keratoconjunctivitis as a result of clinical examination by adapting asepsis and antiseptics principles. The swab samples were placed in tubes containing Stuart transport medium (Gülka Kimya, Ankara, Türkiye) and sent to the microbiology laboratory with a cold chain in

Table 1. Primer sequences and amplification conditions used for PCR detection of bacterial agents in swab samples.

Agent	Gene	Oligonucleotide (5'-3')	bp	Denaturation - Annealing - Elongation (35 cycles)	References
<i>Chlamydiaceae</i> spp.	23S rRNA	F: GGGCTAGACACGTGAAACCTA R: ACCGTAATGGGTAGGAGGGGT	356	94°C / 1 min. 59°C / 1 min. 72°C / 1 min.	Nordentoft et al. 2011
<i>C. pecorum</i>	<i>Cpc</i>	F: TTCGACTTCGCTTCTTCTTACGC R: TGAAGACCGAGCAAACCACC	526	94°C / 1 min. 59°C / 1 min. 72°C / 1 min.	Berri et al. 2009
<i>C. psittaci</i>	<i>ompA</i>	F: CACTATGTGGGAAGGTGCTTCA R: CTGCGCCGGATGCTAATGG	76	94°C / 1 min. 60°C / 1 min. 72°C / 1 min.	Pantchev et al. 2009
<i>C. abortus</i>	<i>Pmp 90/91</i>	F: CTCACCATTGTCTCAGGTGGA R: GGCAATCAGGTGCGACAATCT	821	94°C / 1 min. 60°C / 1 min. 72°C / 1 min.	Berri et al. 2009
<i>Mycoplasma</i> spp.	16S r RNA	F: GCTGGCTGTGTGCCTAATACA R: TGCACCATCTGTCCTGTTAACCTC	1013	94°C / 1 min. 65°C / 1 min. 72°C / 1 min.	Lierz et al. 2007
<i>M. conjunctivae</i>	16S rRNA	F: GTATATCTTTAGAGTCCTCGTCTTTCAC R: CAGCGTGCAGGATGAAATCCCTC	750	94°C / 1 min. 65°C / 1 min. 72°C / 1 min.	Giacometti et al. 1999
<i>M. agalactiae</i>	<i>p80</i>	F: AAAGGTGCTTGAGAAATGGC R: GTTGCAGAAGAAAGTCCAATCA	375	94°C / 1 min. 57°C / 1 min. 72°C / 1 min.	Tola et al. 1994
<i>M. ovis</i>	16S rRNA	F: GAACGATGAGTATCCAGCTTGCT R: CTCTCTTACTTTGGTTAATTATTTGTTGGA	1849	94°C / 1 min. 55°C / 1 min. 72°C / 1 min.	Shen et al. 2011

a short period of time. Identification of *Chlamydia* spp., *Mycoplasma* spp. and *M. ovis* were made by PCR using genus- and species-specific primers (Table 1).

DNA Isolation

Genomic DNA to be used in PCR for the identification of the bacterial agents from swab samples was obtained using a commercial kit (MG-BSDNA-01-100, Hibrigen, Türkiye). The recommendations of the manufacturer were taken into consideration during the application of the kit.

Amplification

Commercial mastermix (2X PCR Mastermix, ABT®, Ankara, Türkiye) was used for the preparation of the PCR mixture. For the optimisation of the mixture, 5 µl of genomic DNA and 1.5 µl of each primer (10 µM) were added in 12.5 µl of mastermix and the total volume was completed to 25 µl with PCR water. Amplification was optimized according to the recommendations of the manufacturers of the mastermix and primers (Table 1). Thus, while the PCR mixture was kept at 94°C for 10 min for pre-denaturation, the final

elongation was applied at 72°C for 15 min. In the test, DNA-free PCR water was used as a negative control. As the positive control, DNA isolated from *M. agalactiae* ATCC 35890 and *C. abortus* (wild strain) supplied from the culture collection of the Department of Microbiology was used.

Electrophoresis

Amplicons obtained as a result of PCR were exposed to electrophoresis (80 V, 1.5 hr) in agarose gel containing gel red. They were compared with the DNA marker (100 bp Plus Opti-DNA Marker G193, ABM, Canada) and examined in a gel imaging system (Gen-Box ImagER, Ankara, Türkiye).

Statistical analysis

The possible correlation between the identified agents and animal's species, age, sex, and stage of the clinical findings in the animals with IKC findings was analysed using Fisher's Exact test method in statistical software. The value of $p \leq 0.05$ was accepted as statistically significant.

Table 2. Distribution of age, sex and infectious keratoconjunctivitis (IKC) stage of sheep with findings in terms of districts.

		District				
		City Centre	Tillo	Şirvan	Kurtalan	Eruh
Sheep (n=163)						
Age	0-1	-	-	18 (40.90%)	45 (78.94%)	10 (33.33)
	>1	32 (100%)	-	26 (59.09%)	12 (21.05%)	20 (66.66)
Sex	M	4 (12.5%)	-	11 (25%)	25 (43.85%)	3 (10)
	F	28 (87.5%)	-	33 (75%)	32 (56.14%)	27 (90)
IKC Stage	1	13 (40.62%)	-	10 (22.75%)	1 (1.75%)	9 (30)
	2	17 (53.12%)	-	18 (40.90%)	26 (45.61%)	13 (43.33)
	3	2 (6.25%)	-	14 (31.81%)	25 (43.85%)	5 (16.66)
	4	-	-	2 (4.54%)	5 (8.77%)	3 (10)
Eye	R	23 (71.87%)	-	24 (54.54%)	9 (15.78%)	22 (73.33)
	L	9 (28.12%)	-	20 (45.45%)	3 (5.26%)	8 (26.66)
	R/L	-	-	-	45 (78.94%)	-
Goat (n=100)						
Age	0-1	10 (100%)	61 (100%)	12 (42.85%)	-	-
	>1	-	-	16 (57.14%)	1 (100%)	-
Sex	M	-	24 (39.34%)	5 (17.85%)	-	-
	F	10 (100%)	37 (60.65%)	23 (82.14%)	1 (100%)	-
IKC Stage	1	9 (90%)	9 (14.75%)	5 (17.85%)	-	-
	2	1 (10%)	22 (36.06%)	12 (42.85%)	-	-
	3	-	15 (24.59%)	6 (21.42%)	-	-
	4	-	15 (24.59%)	5 (17.85%)	1 (100%)	-
Eye	R	8 (80%)	30 (49.18%)	13 (46.42%)	1 (100%)	-
	L	2 (20%)	25 (40.98%)	15 (53.57%)	-	-
	R/L	-	6 (9.83%)	-	-	-

M – Male, F – Female, R – Right, L – Left

Treatment

A single dose of oxytetracycline at a dose of 20 mg/kg was recommended in all animals with IKC findings.

Results

Clinical examination of 632 sheep and 692 goats raised in a total of 13 enterprises in the city centre of Siirt province and Tillo, Şirvan, Kurtalan and Eruh districts was performed in terms of IKC. As a result of the clinical examination, IKC was detected in a total of 263 (19.86%) animals. While it was determined that 163 (61.97%) of the animals with IKC were sheep and 100 (38.02%) were goats, the IKC determination rate was higher in sheep than goats ($p < 0.05$). Forty-two (15.96%) of the cases were detected in the city centre of Siirt province, 61 (23.19%) in Tillo district, 72 (27.37%) in Şirvan district, 58 (22.05%) in Kurtalan district, and 30 (11.40%) in Eruh district (Table 2).

It was determined that 146 (55.51%) of the clinically sick animals were between 0 and 1 year old and 117 (44.48%) animals were older than 1 year. Of the animals, 72 (27.37%) were male and 191 (72.62%) were female. Clinical examination showed that 56 (21.29%), 109 (41.44%), 67 (25.47%), and 31 (11.78%) of the animals had stage 1, 2, 3, and 4 IKC, respectively (Fig. 1). Additionally, 130 (49.42%) and 82 (31.17%) of the animals had IKC in the right and left eyes, respectively, and 51 (19.39%) had findings in both right and left eyes.

While 5 (1.90%) of the swab samples taken from the animals with IKC were positive for *Mycoplasma* spp. by PCR using genus-specific primers, *Chlamydia* spp. was not detected in the samples. Three (60%) of the *Mycoplasma* spp. identified by genus-specific PCR were identified as *M. conjunctivae*. Six (2.28%) of the IKC cases were positive for *M. ovis* by PCR using species-specific primers (Fig. 2, Table 3).

When the distribution of bacterial agents causing IKC cases in small ruminants was analysed according to the stage of IKC; 1 (1.78%) of the stage 1 cases was



Fig. 1. Sheep with (IKC) findings after clinical examination (left: Stage 3 IKC, right: Stage 4 IKC).

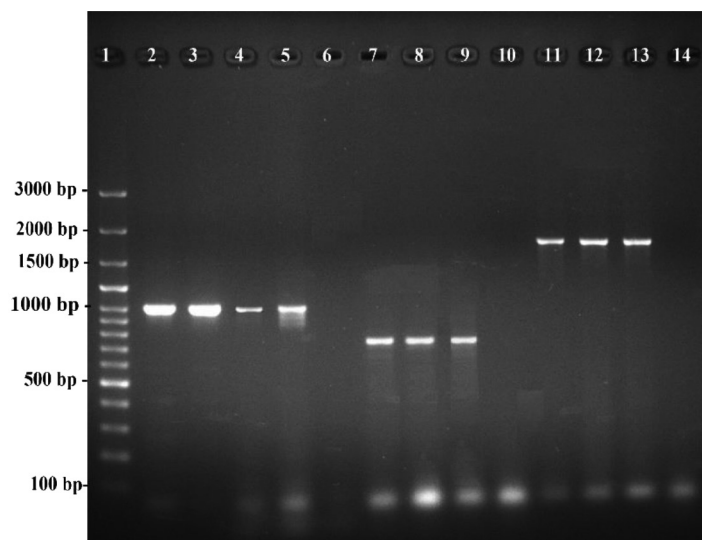


Fig. 2. Image of the amplicons on agarose gel [1: 100 bp DNA ladder, 2: *Mycoplasma agalactiae* ATCC 35890, 3-5: *Mycoplasma* spp. positive sample (1013 bp), 6: Negative control, 7-9: *Mycoplasma conjunctivae* positive samples (750 bp), 10: Negative control, 11-13: *Moraxella ovis* positive samples (1849 bp)].

identified as *Mycoplasma* spp., and 1 (1.78%) as *M. ovis*, 2 (1.83%) of the stage 2 cases were identified as *Mycoplasma* spp. and 3 (2.75%) as *M. ovis*, and 2 (2.98%) of the stage 3 cases were identified as *Mycoplasma* spp. and 2 (2.98%) as *M. ovis*. Table 4 shows the distribution of the bacterial agents identified according to the animals' sex, age, and IKC stage, and the districts.

In the assessment, no statistically significant correlation was obtained regarding the distribution of the bacterial agents identified in terms of the animals' species, sex, age, and IKC stage.

Table 3. Distribution of bacterial agents in sheep with IKC.

	n	<i>Mycoplasma spp.</i>	<i>M. conjunctivae</i>	<i>M. ovis</i>
		positive	positive	positive
Sheep	163	3 (1.84%)	2 (1.22%)	5 (3.06%)
Goat	100	2 (2%)	1 (1%)	1 (1%)
Total	263	5(1.90%)	3(1.14%)	6(2.28%)

Table 4. Distribution of bacterial agents identified according to the sheep' sex, age, and IKC stage, and the districts.

	Animal's				
	District	Species	Sex	Age (years)	IKC Stage
<i>M. conjunctivae</i>	Tillo	Goat	Female	0-1	3
<i>M. conjunctivae</i>	Şirvan	Sheep	Female	>1	2
<i>M. conjunctivae</i>	Şirvan	Sheep	Female	>1	2
<i>Mycoplasma spp.</i>	Tillo	Goat	Male	0-1	3
<i>Mycoplasma spp.</i>	Şirvan	Sheep	Male	>1	1
<i>M. ovis</i>	Şirvan	Goat	Female	0-1	2
<i>M. ovis</i>	Eruh	Sheep	Female	>1	2
<i>M. ovis</i>	Eruh	Sheep	Female	>1	1
<i>M. ovis</i>	Eruh	Sheep	Female	0-1	2
<i>M. ovis</i>	Eruh	Sheep	Female	0-1	3
<i>M. ovis</i>	Eruh	Sheep	Female	0-1	3

Discussion

Although IKC cases vary according to their stage, they cause serious clinical findings in small ruminants and lead to economic losses in herds (Akerstedt and Hofshagen 2004, Gelormini et al. 2017, Işık et al. 2018). It has been reported that *Chlamydia* spp. and *Mycoplasma* spp. isolates are endemic in sheep and goats around the world. The need for complex environments for the growth of the agents and a long incubation period prevents their diagnosis via routine laboratory tests (Pantchev et al. 2010, Nordentoft et al. 2011, Quiin et al. 2011). Therefore, the aim of the study was to detect the agents directly by PCR from eye swab samples obtained from the animals clinically diagnosed with keratoconjunctivitis.

In the present study, a total of 19.86% of 632 sheep and 692 goats were found to have IKC as a result of clinical examination. In the evaluation, it was observed that the rate of IKC detection was higher in sheep compared to goats ($p < 0.05$). In a previous study conducted in the region where the study was conducted, it was determined that the rate of IKC detection was higher in sheep than goats (Işık and Durmuş 2022).

Similarly, in a study conducted by Fernández et al. (2017) on 176 sheep and 158 goats in Pakistan, they highlighted that number of sheep showing IKC findings was significantly higher than the number of goats.

In the study conducted by Naglic et al. (2000) in Croatia, they reported that while *Mycoplasma* spp. was isolated in 48% of the sheep samples, *M. ovis* was isolated in 58%. It was stated that 12 of 42 *Mycoplasma* spp. strains isolated were *M. conjunctivae*. Akerstedt and Holshagen (2004) reported that they isolated *M. ovis* in 28% and *Mycoplasma* spp. in 3% of the samples taken from sheep with infectious keratoconjunctivitis. In the study conducted by Fernández-Aguilar et al. (2013) in Spain, they identified *M. conjunctivae* in 18% of the eye swab samples taken from sheep and goats. In a similar study conducted in New Zealand, *M. conjunctivae* was observed in 39.21% of eye swab samples taken from small ruminants (Motha et al. 2003). Shahzad et al. (2013) identified *M. conjunctivae* by PCR in 59% of 36 eye swab samples taken from sheep with IKC findings in Pakistan. In a study conducted in Malaysia, it was reported that *M. ovis* was identified in 30% of eye swab samples taken from 60 goats (Abdullah et al. 2015). In a study

conducted in Turkey, it was reported that *M. ovis* was identified in 85.71% and *M. conjunctivae* in 7.14% of eye swab samples taken from 42 animals in a sheep farm in the Kars region (Gülmez Sağlam et al. 2018).

In the present study, *Mycoplasma* spp. was identified in 1.90%, *M. conjunctivae* in 1.14% and *M. ovis* in 2.28% of a total of 263 sheep and goats with IKC findings as a result of clinical examination. In contrast to this study, it was observed in the results that the identification rate of *Mycoplasma* spp. in cases of IKC in small ruminants can be quite high (30-50%) (Naglic et al. 2000, Gupta et al. 2014, Fernández-Aguilar et al. 2017). On the other hand, similar to the present study, Akerstedt and Holshagen (2004) determined the rate of *Mycoplasma* spp. as 3% in their study. Although the identification rate of *M. conjunctivae* from eye swab samples of small ruminants varied between 13% and 60% in the related studies, *M. conjunctivae* was identified from a limited number of samples in the present study. It was reported that the rate of *M. ovis* isolates, which can be isolated from the conjunctival flora of both healthy and sick animals, can increase to 85% in samples taken from sheep and goats with IKC (Naglic et al. 2000, Akerstedt and Holshagen 2004, Abdullah et al. 2015, Gülmez Sağlam et al. 2018); whereas, in the present study, *M. ovis* was detected in 6 (2.28%) of the animals with eye lesions.

Although *Chlamydia* spp. strains cause abortion in small ruminants (Gülaydın et al. 2023), it is reported to be determined from the eye swab samples taken from healthy animals and/or those with conjunctivitis symptoms. Thus, Polkinghorne et al. (2009) examined the samples taken from a total of 128 healthy sheep and sheep with conjunctivitis symptoms for the presence of *Chlamydia* spp. Accordingly, they reported that *C. abortus* was identified in 15.29%, *C. pecorum* in 4.70% and *C. suis* in 5.88% of the eye swab samples taken from 85 sick animals. In the study, it was also reported that *C. abortus* was identified from 13 and *C. suis* from 2 of 43 healthy animals and enzootic abortion cases were observed in the previous year in the herd where *C. abortus* was detected. In their study, Gupta et al. (2014) investigated the presence of *Chlamydia* spp. and *Mycoplasma* spp. in the samples taken from sheep and goats with ocular disease findings; they reported the identification of *Chlamydia* spp. in 77.41% and *Mycoplasma* spp. in 41.93% of sheep samples, and *Chlamydia* spp. in 14.29% and *Mycoplasma* spp. in 14.29% of goat samples. However, it was emphasised in the study that mostly *C. abortus*, *C. psittaci*, *M. arginini*, and *M. hyorhinis* agents were identified, and it was also noted that more than one agent was detected simultaneously in some cases. In another study conducted to determine the aetiology of IKC cases

observed in sheep and goats, it was reported that *Chlamydia* spp. was identified in 16.7% and *M. conjunctivae* in 33.3% of the swab samples taken from sick sheep and *M. conjunctivae* in 50% of the swab samples taken from sick goats. It was reported that *Chlamydia* spp. and *M. conjunctivae* were identified together in 2 of 12 cases observed in sheep (Fernández-Aguilar et al. 2017). Jelocnik et al. (2019) reported that *C. psittaci* was identified in 5.6% and *C. pecorum* in 28.5% of the swab samples taken from sheep with IKC findings. *C. abortus* was not identified in the samples examined in the study.

While the identification rate of *Chlamydia* spp. in small ruminants with IKC findings ranges from 5% to 80% (Polkinghorne et al. 2009, Fernández-Aguilar et al. 2017, Jelocnik et al. 2019), *Chlamydia* spp. was not identified from eye swab samples in this study. In addition, in contrast to other studies (Gupta et al. 2014, Fernández-Aguilar et al. 2017), more than one bacterial species were not identified in the same sample.

While conjunctival samples were mostly taken from herds with IKC outbreaks in other studies, the fact that the majority of the samples (62.73%) in the present study were taken from 1st and 2nd stage conjunctivitis cases may have caused the number of bacterial agents detected to be low. Furthermore, other microorganisms whose presence was out of the range of this study, might have caused IKC cases. Additionally, the number of bacteria in swab samples might have been under the detection rate of the conventional PCR method. It is known that swabs are useful for collecting DNA and/or bacteria from the lesion area, but poor at releasing the DNA on extraction procedure. Moreover, DNA extraction methods can affect the yield of DNA amount (Adamowicz et al. 2014, Gray et al. 2023). Therefore, false negative PCR results due to the extraction method might have caused discrepancies in detection rates of the bacteria. Also, it was thought that the difference in the identification rates of bacterial agents in different studies may vary according to the number of samples used, the resistance developed due to the hereditary characteristics of the animals, and geographical conditions.

In another study, it was determined that while 4 (11.8%) of 34 sheep positive for *M. conjunctivae* showed signs of tear discharge, 30 (88.2%) had no clinical signs at the time of sampling. In goats, 1 (6.66%) showed severe IKC symptoms (bilateral corneal perforation) of *M. conjunctivae*, while the remaining 14 (93.3%) did not show any clinical signs (Fernández et al. 2017). In the present study, it was determined that the distribution of bacterial agents causing IKC cases in small ruminants differed according to IKC findings and there was no statistically sig-

nificant correlation between the stage of IKC and the bacterial agents identified.

Numerous methods are used in the treatment of IKC in sheep and goats (Işık et al. 2018). Among such methods, systemic single dose oxytetracycline, tylosin, streptomycin, and chlortetracycline applications are given. Locally, tobramycin, aureomycin (chlortetracycline) and gentamicin preparations are said to be effective (Hosie 2007, Boileau et al. 2012). It was also emphasised that the addition of 150-200 mg oxytetracycline per animal per day to the feed in sheep reduced the severity and incidence of the disease (Townsend 2008). In an in vitro study on *M. conjunctivae*, tylosin, oxytetracycline, streptomycin, and chlortetracycline antibiotics were reported to be effective (Egwu 1992). Likewise, in another study conducted in animals with *M. conjunctivae* isolated from IKC, it was reported that 0.5% gentamicin was administered morning and evening for 5 days and successful results were obtained (Shahzad et al. 2013). In the present study, in the light of the literature, it is recommended that a single dose of oxytetracycline (20 mg/kg) injection be administered to all 263 animals with IKC because it is easy and economical to apply in farms. However, treatment and recovery processes could not be followed because of the nomadic animal husbandry system practiced in the region.

Consequently, the current study showed that the rate of IKC in sheep and goats raised in and around Siirt province was 19.86% and the rate of IKC in sheep was higher compared to goats. It was revealed that *Mycoplasma* spp. and *M. ovis* strains can be rapidly detected by PCR from IKC cases in small ruminants. Other bacterial and/or viral agents causing IKC cases in small ruminants in the region should be investigated in future studies. In addition, in-vitro antimicrobial susceptibilities of bacterial agents to be obtained from IKC cases by conventional methods should be determined and treatment processes should be evaluated accordingly. It is suggested that the results obtained will contribute to the studies regarding the protection and control of IKC cases in sheep and goats raised in the region.

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References

Abdullah FF, Naidu NR, Sadiq MA, Abba Y, Tijjani A, Mohammed K, Chung EL, Norsidin MJ, Lila MA,

- Haron AW, Aziz A, Saharee OA (2015) Prevalence of *Moraxella ovis* infection in goats under the Ladang Angkat Programme, Universiti Putra Malaysia: A Cross-Sectional Study. IOSR-JAVS 8: 99-102.
- Adamowicz MS, Stasulli DM, Sobestanovich EM, Bille TW (2014) Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. PLoS ONE 9: e116351.
- Aguilar XF, Cabezón Ó, Marco I, Mentaberre G, Frey J, Lavín S, López-Olvera JR (2013) *Mycoplasma conjunctivae* in domestic small ruminants from high mountain habitats in Northern Spain. BMC Vet Res 9: 253.
- Åkerstedt J, Hofshagen M (2004) Bacteriological investigation of infectious keratoconjunctivitis in Norwegian sheep. Acta Vet Scand 45: 19-26.
- Akın F, Samsar E (2005) Göz hastalıkları. Medipres Matbaacılık Ltd. Şti, Malatya, pp 120-121.
- Berri M, Rekiki A, Boumedine KS, Rodolakis A (2009) Simultaneous differential detection of *Chlamydophila abortus*, *Chlamydophila pecorum* and *Coxiella burnetii* from aborted ruminant's clinical samples using multiplex PCR. BMC Microbiol 9(1): 130.
- Boileau MJ, Gilmour MA (2012) Diseases of the eye. In: Pugh DG, Baird AN (eds) Sheep and goat medicine. Elsevier Inc, Missouri.
- Cakir L, Gümüşsoy KS, Kutsal O, Tunc AS (2014) Evaluation of brush cytology (cytospin technique) and cultural results in the diagnosis of keratoconjunctivitis in a goat herd. Ankara Univ Vet Fak Derg 61: 35-41.
- Egwu GO (1992) In vitro antibiotic-sensitivity of *Mycoplasma conjunctivae* and some bacterial species causing ovine infectious kerato-conjunctivitis. Small Rumin Res 7: 85-92.
- Fernández-Aguilar X, Cabezón O, Marco I, Mentaberre G, Frey J, Lavín S, López-Olvera JR (2013) *Mycoplasma conjunctivae* in domestic small ruminants from high mountain habitats in Northern Spain. BMC Vet Res 9: 253.
- Fernández-Aguilar X, Rossi L, Cabezón O, Giorgino A, Llopis VI, Frey J, López-Olvera JR (2017) Infectious keratoconjunctivitis and occurrence of *Mycoplasma conjunctivae* and *Chlamydiaceae* in small domestic ruminants from Central Karakoram, Pakistan. Vet Rec 181: 237-244.
- Gelormini G, Gauthier D, Vilei EM, Crampe JP, Frey J, Ryser-Degiorgis MP (2017) Infectious keratoconjunctivitis in wild Caprinae: merging field observations and molecular analyses sheds light on factors shaping outbreak dynamics. BMC Vet Res 13: 67.
- Giacometti M, Janovsky M, Belloy L, Frey J (2002) Infectious keratoconjunctivitis of ibex, chamois and other Caprinae. Rev Sci Tech 21: 335-345.
- Giacometti M, Nicolet J, Johansson KE, Naglic T, Degiorgis MP, Frey J (1999) Detection and identification of *Mycoplasma conjunctivae* in infectious keratoconjunctivitis by PCR based on the 16S rRNA Gene. Zentralbl Veterinarmed B 46: 173-180.
- Gray A, Kuffel A, Daeid NN (2023) An improved rapid method for DNA recovery from cotton swabs. Forensic Sci Int Genet 64: 102848.
- Gülaydin Ö, Öztürk C, Ekin İH, İlhan Z, İlhan F (2023) Investigation of selected bacterial agents causing sheep abortion in the Van Province by RT-PCR and histopathological methods. Acta Vet Brno 92: 69-77.
- Gülmez Sağlam A, Erkilic EE, Büyük F, Kırmızıgül AH,

- Gökçe G, Balyen L, Akyüz E, Aydın U, Özba B, Otlı S (2018) *Moraxella ovis* and *Mycoplasma conjunctivae* isolation from an ovine infectious keratoconjunctivitis outbreak and fortified treatment approaches. *Kafkas Univ Vet Fak Derg* 24: 551-556.
- Gupta S, Chahota R, Bhardwaj B, Priyanka P, Verma S, Sharma M (2014) Identification of *Chlamydiae* and *Mycoplasma* species in ruminants with ocular infections. *Lett Appl Microbiol* 60: 135-139.
- Hosie BD (2007) Ocular diseases. In: Aitken ID (ed) *Diseases of sheep*. Blackwell Publishing, USA
- Işık R, Durmuş AS (2020) Prevalence of eye diseases in the sheep and goats in Siirt and its surrounding region. *FU Sag Bil Vet Derg* 34: 127-132.
- Işık Y, Ceylan C, Erol M (2018) Infectious keratoconjunctivitis of small ruminants. *BAUN Sag Bil Derg* 7: 112-116.
- Jelocnik M, Laurence M, Murdoch FR, Polkinghorne A (2019) Detection of *Chlamydiaceae* in ocular swabs from Australian pre-export feedlot sheep. *Aust Vet J* 97: 401-403.
- Karthik K, Manimaran K, Mahaprabhu R, Shoba K (2017) Isolation of *Moraxella* sp. from cases of keratoconjunctivitis in an organized sheep farm of India. *Open J Vet Med* 7: 138-143.
- Lierz M, Hagen N, Harcourt-Brown N, Hernandez-Divers SJ, Lüschoff D, Hafez HM (2007) Prevalence of mycoplasmas in eggs from birds of prey using culture and a genus-specific mycoplasma polymerase chain reaction. *Avian Pathol* 36: 145-150.
- Loison A, Gaillard JM, Jullien JM (1996) Demographic patterns after an epizootic of keratoconjunctivitis in a chamois population. *J Wildl Manage* 60: 517-527.
- Mayer D, Degiorgis MP, Meier W, Nicolet J, Giacometti M (1997) Lesions associated with infectious keratoconjunctivitis in alpine ibex. *J Wildl Dis* 33: 413-419.
- Motha MXJ, Frey J, Hansen MF, Jamaludin R, Tham KM (2003) Detection of *Mycoplasma conjunctivae* in sheep affected with conjunctivitis and infectious keratoconjunctivitis. *N Z Vet J* 51: 186-190.
- Naglić T, Hajsig D, Frey J, Šeol B, Busch K, Lojkić M (2000) Epidemiological and microbiological study of an outbreak of infectious keratoconjunctivitis in sheep. *Vet Rec* 147: 72-75.
- Nordentoft S, Kabell S, Pedersen K (2011) Real-time detection and identification of *Chlamydia* species in veterinary specimens by using SYBR green-based PCR assays. *Appl Environ Microbiol* 77: 6323-6330.
- Pantchev A, Sting R, Bauerfeind R, Tyczka J, Sachse K (2009) New real-time PCR tests for species-specific detection of *Chlamydia psittaci* and *Chlamydia abortus* from tissue samples. *Vet J* 181: 145-150.
- Pantchev A, Sting R, Bauerfeind R, Tyczka J, Sachse K (2010) Detection of all *Chlamydia* and *Chlamydia* spp. of veterinary interest using species-specific real-time PCR assays. *Comp Immunol Microbiol Infect Dis* 33: 473-484.
- Polkinghorne A, Borel N, Becker A, Lu ZH, Zimmermann DR, Brugnera E, Pospischil A, Vaughan L (2009) Molecular evidence for chlamydial infections in the eyes of sheep. *Vet Microbiol* 135: 142-146.
- Quinn PJ, Markey BK, Leonard FC, FitzPatrick ES, Fanning S, Hartigan PJ (2011) *Veterinary microbiology and microbial disease*. 2nd ed., John Wiley & Sons Ltd., UK, pp: 300-308.
- Rodolakis A, Laroucau K (2015) *Chlamydiaceae* and chlamydial infections in sheep or goats. *Vet Microbiol* 181: 107-118.
- Romano JS, Leijon M, Hagström Å, Jinnerot T, Rockström UK, Tryland M (2019) *Chlamydia pecorum* associated with an outbreak of infectious keratoconjunctivitis in semi-domesticated reindeer in Sweden. *Front Vet Sci* 6: 14.
- Shahzad W, Munir R, Rana MY, Ahmad R, Khan MS, Akbar G, Mohammad I, Mehmood F (2013) Prevalence, molecular diagnosis and treatment of *Mycoplasma conjunctivae* isolated from infectious keratoconjunctivitis affected Lohi sheep maintained at Livestock Experiment Station, Bahadurnagar, Okara, Pakistan. *Trop Anim Health Prod* 45: 737-742.
- Shen HG, Gould S, Kinyon J, Opriessnig T, O'Connor AM (2011) Development and evaluation of a multiplex real-time PCR assay for the detection and differentiation of *Moraxella bovis*, *Moraxella bovoculi* and *Moraxella ovis* in pure culture isolates and lacrimal swabs collected from conventionally raised cattle. *J Appl Microbiol* 111: 1037-1043.
- Tola S, Idini G, Manunta D, Galleri G, Angioi A, Rocchigiani AM, Leori G (1996) Rapid and specific detection of *Mycoplasma agalactiae* by polymerase chain reaction. *Vet Microbiol* 51: 77-84.
- Townsend WM (2008) Food and fiberproducing animal ophthalmology. In: Kirk NG (ed) *Essentials of veterinary ophthalmology*. 2nd ed., Blackwell Publishing, UK, pp 379-418.