

Prevalence of *Mycoplasma* spp., *Chlamydia* spp., and *Moraxella* species in ocular samples from European bison (*Bison bonasus*) and their association with infectious keratoconjunctivitis

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Abstract

Background.

Infectious keratoconjunctivitis (IKC) is a multifactorial ocular disease affecting domestic and wild ruminants. *Mycoplasma*, *Chlamydia* and *Moraxella* spp. have been associated with ocular disease in various ruminant species, but their occurrence and potential role in IKC in European bison (*Bison bonasus*) remain poorly understood. This study aimed to determine the occurrence of *Mycoplasma* spp., *Chlamydia* spp., *Moraxella bovoculi* and *Moraxella bovis* in ocular samples from European bison and to assess their association with the occurrence and severity of ocular lesions.

Results.

Ocular samples were collected from 55 European bison in Poland between 2023 and 2025 and examined using real-time PCR. At least one of the investigated bacterial pathogens was detected in 26/55 (47.3%) bison. *Mycoplasma* spp. and *M. bovoculi* were each detected in 13/55 (23.6%) animals, while *Chlamydia* spp. was detected in 3/55 (5.5%); *M. bovis* was not detected. Ocular lesions were observed in 42/55 (76.4%) bison. Neither the occurrence nor severity of ocular lesions was significantly associated with the detection of the examined bacteria. Histopathologically, conjunctival lesions were characterized predominantly by mononuclear inflammatory infiltrates, while corneal lesions were generally mild and mainly involved stromal edema and epithelial and endothelial alterations.

Conclusions. Mycoplasma

spp., *M. bovoculi* and *Chlamydia* spp. were detected in ocular samples from European bison, with *Mycoplasma* spp. and *M. bovoculi* being the most frequently detected pathogens. However, their presence was not significantly associated with the occurrence or severity of ocular lesions. These findings indicate that the investigated bacteria may be components of the complex ocular microbiological landscape in European bison, but their specific contribution to the pathogenesis of IKC remains unclear and warrants further investigation.

Background

Infectious keratoconjunctivitis (IKC) is an ocular disease affecting domestic and wild ruminants worldwide. Clinical manifestations range from conjunctivitis, excessive lacrimation and corneal opacity to corneal ulceration, impaired vision and, in severe cases, blindness. Although mortality is generally low, IKC may cause considerable morbidity and can also occur in a subclinical form, allowing infectious agents to persist and spread within animal populations [1–3].

The etiology of IKC is considered multifactorial and involves interactions among viral, bacterial and parasitic pathogens together with environmental and host-related factors [3, 4]. Bacteria commonly associated with IKC include *Mycoplasma conjunctivae*, the principal etiological agent of IKC in wild Caprinae and domestic sheep and goats, *Chlamydia* spp., particularly *Chlamydia* (*Ch.*) *pecorum*, and *Moraxella* spp., including *Moraxella* (*M.*) *bovis* and *Moraxella* (*M.*) *bovoculi*, although their pathogenic significance differs among host species and epidemiological settings [1, 4–11]. Viral pathogens, particularly cervid herpesvirus 2 (CvHV2), as well as parasitic ones, like *Thelazia* nematodes have also been implicated in the development of IKC in several wild ruminant species [3, 12].

An outbreak of IKC has affected European bison (*Bison bonasus*) in Poland since 2019. The disease primarily occurs in the Lowland-Caucasian genetic line inhabiting the Bieszczady Mountains, where approximately 15% of the population has developed clinical signs of IKC [13]. Previous studies demonstrated a high prevalence of *Thelazia* spp. in affected animals; however, parasitic infection alone could not explain the occurrence and severity of ocular lesions [12–14]. Moreover, characterization of the ocular surface microbiota identified several bacterial taxa potentially associated with IKC, but failed to detect the bacterial genera most commonly implicated in the disease in other ruminant species [15].

Therefore, the present study aimed to investigate the prevalence of *Mycoplasma* spp., *Chlamydia* spp., *M. bovis* and *M. bovoculi* in ocular samples collected from European bison and to evaluate their potential association with the occurrence and severity of infectious keratoconjunctivitis.

Results

Study population

The study population included 55 European bison, 33 males (60.0%) and 22 females (40.0%), aged between 3 months and 24 years (median: 10 years, IQR: 3–13 years) (data available for 53 bison). Forty one of 55 bison (74.5%) were from Bieszczady Mountains and belonged to the Lowland-Caucasian genetic line, while 14 bison (25.5%) were from north-eastern and south-western Poland and belonged to the Lowland genetic line.

In the Lowland-Caucasian genetic line, a male-to-female ratio was 21:20 while the Lowland genetic line included significantly more males (12/14; 86%) than females (2/14; 14%) ($p = 0.017$).

Age did not differ significantly between males and females ($p = 0.730$) or between genetic lines ($p = 0.262$). Body weight (data available for 43 bison) ranged between 70 and 855 kg (median: 430 kg, IQR: 275–702 kg). Males ($n = 24$; median: 681 kg, IQR: 515–753 kg) were significantly heavier than females ($n = 19$; median: 306 kg, IQR: 225–376 kg; $p = 0.001$). Body weight did not differ significantly between genetic lines ($p = 0.707$).

Significantly more bison were obtained during vegetation season ($n = 35$; 63.6%) than during winter ($n = 20$; 36.4%) ($p = 0.042$).

Detection of bacteria

In 35 of the 55 European bison (63.6%), samples from both eyes were analysed by real-time PCR, whereas in the remaining 20 animals (33.4%) only one eye was tested. In total, 90 ocular samples were examined for the presence of *Mycoplasma* spp., *M. bovoculi*, *M. bovis*, and *Chlamydia* spp., including 44 samples from the right eye and 46 from the left eye.

Overall, at least one bacterial pathogen was detected in 26 of the 55 European bison (47.3%; CI 95%: 34.7% – 60.2%). *Mycoplasma* spp. and *M. bovoculi* were the most frequently detected bacteria, each identified in 13 of 55 European bison (23.6%). Both microorganisms were detected significantly more often than *Chlamydia* spp. (3/55; 5.5%; $p = 0.021$). *M. bovis* was not detected in any of the examined animals. Detailed results are presented in Table 1.

Co-occurrence were uncommon and occurred in only 3 of 55 European bison (5.5%): in two individuals *Mycoplasma* spp. and *M. bovoculi* while in one individual *Mycoplasma* spp. and *Chlamydia* spp. were detected simultaneously. No animal was positive for more than two microorganisms.

The frequency of detection of each bacterium did not differ significantly between the right and left eyes ($p = 0.999$ for all three microorganisms). Among 35 European bison from which both eyes were sampled, bilateral detection of the same microorganism was uncommon. Overall, only 10 of 35 animals (28.6%) had at least one microorganism detected in both eyes (Table 1).

Table 1

Prevalence of three analyzed groups of bacteria (*Mycoplasma* spp., *Moraxella* spp., and *Chlamydia* spp.)

Bacteria	Number of positive European bison	Prevalence (CI 95%)	Right eye (n = 44)	Left eye (n = 46)	Only European bison with both eyes examined (n = 35)
<i>Mycoplasma</i> spp.	13 / 55	23.6% (14.4% – 36.3%)	10 / 44 (22.7%)	9 / 46 (19.6%)	6 / 35 (17.1%)
<i>Chlamydia</i> spp.	3 / 55	5.5% (1.9% – 14.9%)	1 / 44 (2.3%)	3 / 46 (6.5%)	1 / 35 (2.9%)
<i>Moraxella bovoculi</i>	13 / 55	23.6% (14.4% – 36.3%)	8 / 44 (18.2%)	9 / 46 (19.6%)	4 / 35 (11.4%)
Any bacterium	26 / 55	47.3% (34.7% – 60.2%)	17 / 44 (38.6%)	19 / 46 (41.3%)	10 / 35 (28.6%)

Factors associated with bacterial infections

European bison positive for analyzed bacteria, were significantly younger ($n = 26$; median: 6 years; IQR: 3–12 years) than animals with no bacteria detected ($n = 27$; median: 12 years; IQR: 5–15 years; $p = 0.033$). The most optimal cut-off value for age was ≤ 13 years, i.e. any examined microorganism was detected significantly more often in European bison at the age ≤ 13 years than in individuals older than 13 years ($p = 0.008$). Moreover, European bison positive for analyzed bacteria had lower body weight ($n = 21$; median: 300 kg; IQR: 220–664 kg) than negative individuals ($n = 22$; median: 620 kg; IQR: 360–702 kg) and this difference was close to statistical significance ($p = 0.063$). No other significant associations between the presence of bacteria and genetic line ($p = 0.701$), sex ($p = 0.741$), or season in which obtained ($p = 0.798$) were observed (Table 2).

Table 2

Factors associated with the presence of any of the three analyzed groups of bacteria (*Mycoplasma* spp., *Moraxella* spp., and *Chlamydia* spp.)

Factor	Category	Number (%) of European bison with any bacteria in the category	p-value	Odds ratio (CI 95%)
Season	Winter (November to April)	9 / 20 (45.0%)	0.798	Reference category
	Vegetation (May to October)	17 / 35 (48.6%)		1.15 (0.38–3.48)
Genetic line	Lowland – Caucasian	20 / 41 (48.8%)	0.701	Reference category
	Lowland	6 / 14 (42.9%)		0.79 (0.23–2.68)
Sex	Female	11 / 22 (50.0%)	0.741	Reference category
	Male	15 / 33 (45.5%)		0.83 (0.28–2.46)
Age [years] ^b	> 13 years	2 / 12 (16.7%)	0.008	Reference category
	≤ 13 years	24 / 41 (58.5%)		7.06 (1.37–36.4)
^a presented as median, interquartile range (IQR), and range				
^b categorization according to the most optimal cut-off of ≤ 13 years				

Mycoplasma spp. was detected significantly more frequently in European bison representing the Lowland-Caucasian genetic line (13/41; 31.7%) than in those belonging to the Lowland genetic line (0/14; 0%; $p = 0.003$).

Animals positive for *Mycoplasma* spp. were significantly younger ($n = 13$; median: 5 years; IQR: 3–12 years) than those which tested negative ($n = 40$; median: 12 years; IQR: 4–14 years). The optimal age cut-off value was ≤ 13 years, and *Mycoplasma* spp. was detected significantly more frequently in European bison aged ≤ 13 years (13/41; 31.7%) than in animals older than 13 years (0/12; 0%; $p = 0.020$). European bison positive for *Mycoplasma* spp. had also significantly lower body weight ($n = 12$; median: 288 kg; 215–458) than negative animals ($n = 31$; median: 600 kg; IQR: 340–731; $p = 0.017$). The optimal cut-off value for body weight was 310 kg, and *Mycoplasma* spp. was detected significantly more frequently in European bison weighing ≤ 310 kg (13/41; 31.7%) than in those weighing > 310 kg (0/12; 0%; $p = 0.001$). Detailed results are presented in Supplementary Table S1

No factor was significantly associated with the presence of *M. bovoculi* (Supplementary Table S2) and detection of *Chlamydia* spp. in only 3 individuals precluded analysis of factors associated with the presence of this species.

Ocular lesions

Ocular lesions were observed in 42 of 55 examined European bison (76.4%; CI 95%: 63.7% – 85.6%). Among the affected animals, score 1 was the least common (4/42; 9.5%), whereas scores 2 and 3 occurred with equal frequency (19/42; 45.2% each). Lesions were bilateral in 17 of 55 animals (30.9%) and unilateral in 25 of 55 animals (45.5%; right eye affected in 14 and left eye in 11 animals). Neither the prevalence nor the severity of ocular lesions differed significantly between the right and left eyes ($p = 0.831$ and $p = 0.423$, respectively). The severity of lesions, scored from 0 to 3, is presented in Fig. 1.

The presence of ocular lesions was not significantly associated with the detection of any of the examined bacterial pathogens ($p = 0.755$).

Histopathological examination of the conjunctiva revealed epithelial hyperplasia, exfoliation and, less frequently, epithelial vacuolation. The lamina propria commonly contained mononuclear inflammatory infiltrates composed of lymphocytes, plasma cells and macrophages, with lymphoid follicle formation and perivascular infiltration observed in several specimens. These changes were occasionally accompanied by edema and connective tissue proliferation. The severity of conjunctival lesions varied from minimal to moderate, with more pronounced inflammatory changes characterized by marked mononuclear infiltrates and lymphoid follicle formation observed in some specimens.

Histopathological examination of the cornea most commonly revealed stromal edema, epithelial vacuolation and exfoliation, together with endothelial cell vacuolation and exfoliation. Mononuclear inflammatory infiltrates and stromal vascularization were observed less frequently. Two specimens showed more advanced focal lesions, including epithelial ulceration, stromal connective tissue proliferation and neovascularization; one of these also exhibited prominent inflammatory infiltrates with numerous plasma cells and sub-Descemet inflammation. Other occasional findings included reactive

endothelial changes, focal lymphocytic infiltration near the limbus, subepithelial cyst-like structures and anterior synechiae.

Overall, conjunctival lesions were more consistently characterized by inflammatory changes, whereas corneal lesions were predominantly mild and characterized by edema and epithelial and endothelial alterations. Representative histopathological changes are presented in Fig. 2.

Discussion

Bacteria of the genera *Mycoplasma* and *Chlamydia*, as well as *M. bovoculi*, were detected in almost a half of the examined European bison. To the best of our knowledge, this is the first report of the detection of *Mycoplasma* spp. and *Chlamydia* spp. in ocular samples collected from European bison, whereas *M. bovoculi* has previously been detected only sporadically in this species. These microorganisms have all been associated with IKC in domestic and wild ruminants; however, their pathogenic significance differs substantially among host species and is currently considered to be multifactorial rather than caused by a single pathogen [2–4, 6, 7]. This concept is consistent with our previous investigations on IKC in European bison, in which neither *Thelazia* infection nor any single epidemiological factor could fully explain the occurrence and severity of ocular lesions [12, 13].

Unlike *M. bovis*, which has been experimentally confirmed as the primary bacterial pathogen of infectious bovine keratoconjunctivitis (IBK) [8], the pathogenic role of *M. bovoculi* remains unclear. Although *M. bovoculi* has been isolated from cattle affected by IBK, it is also frequently detected in clinically healthy eyes, supporting its classification as a potential opportunistic pathogen rather than a primary causative agent [4, 6, 7]. In our study, *M. bovoculi* was detected in almost 25% of examined European bison, whereas *M. bovis* was not detected in any animal at all. The detection of *M. bovoculi* in a substantial proportion of European bison, together with the absence of *M. bovis*, may suggest that *M. bovoculi* could play a role in the ocular microbiota of European bison; however, its contribution to the development of IKC remains uncertain. Notably, *M. bovoculi* has also been detected during outbreaks of IKC in semi-domesticated reindeer in Scandinavia, where it occurred in association with other pathogens, particularly *Ch. pecorum* and CvHV2 [16].

In our study, *Mycoplasma* spp. was detected in almost 25% of the examined European bison, with a prevalence identical to that of *M. bovoculi*. The detection of *Mycoplasma* spp. was significantly more frequent among bison belonging to the Lowland-Caucasian genetic line. This genetic line is represented by the population inhabiting the Bieszczady Mountains, where the outbreak of IKC reported in European bison occurred [13]. This finding may reflect population-specific differences in exposure to *Mycoplasma* spp. or its transmission dynamics and warrants further investigation. In wild and domestic small ruminants, IKC is primarily associated with *Mycoplasma (M.) conjunctivae*, which is recognized as the principal etiological agent and has been linked to conjunctivitis, keratitis, corneal ulceration, and blindness [1, 5, 10]. In contrast, *M. conjunctivae* has only occasionally been reported in members of the subfamily Bovinae, and its epidemiological significance in these hosts remains uncertain [17]. Moreover,

M. conjunctivae is frequently detected in clinically healthy animals, indicating that infection does not necessarily result in clinical disease [10, 18, 19]. In domestic sheep from northern Spain, the overall prevalence of *M. conjunctivae* was approximately 20%, whereas nearly 90% of PCR-positive animals showed no clinical signs of IKC [10]. Likewise, endemic infections with predominantly subclinical courses have been described in wild Caprinae, with prevalence varying considerably among host populations and epidemiological settings [11]. Recent evidence from a mountain ecosystem demonstrated that *M. conjunctivae* can be maintained through simultaneous epidemiological cycles involving wildlife and livestock, emphasizing the potential importance of host interactions in the transmission and persistence of this pathogen [20]. Because *Mycoplasma* spp. was identified only to the genus level in the present study, the clinical significance of the detected organisms remains uncertain. Species-level identification, together with further epidemiological investigation, will therefore be necessary to determine their distribution, transmission dynamics and potential role in ocular disease in European bison.

Similarly, *Chlamydia* spp. was identified only to the genus level. Ocular infections in domestic and wild ruminants are most frequently associated with *Ch. pecorum*, although other chlamydial species have also been detected in animals with conjunctivitis [21]. In the present study, *Chlamydia* spp. were detected in only three European bison (5.5%), which limits the interpretation of their epidemiological and clinical significance.

No significant association was found between the presence of *Mycoplasma* spp., *Chlamydia* spp. or *M. bovoculi* and either the occurrence or severity of ocular lesions. Similar observations have been reported previously, indicating that these bacteria may occur in clinically healthy animals and may represent components of the normal ocular microbiota or opportunistic pathogens rather than primary etiological agents [4, 6, 10, 11, 21]. Clinical disease is thought to develop when bacterial colonization coincides with additional predisposing factors, including ultraviolet radiation, dust, flies, mechanical trauma, viral infections and impaired host immunity [16]. Co-infections involving several bacterial species, particularly *M. conjunctivae* and *Ch. pecorum*, have been suggested to increase the probability of clinical IKC [21], however mixed bacterial infections were detected only sporadically in the present study.

Most ocular samples were collected from European bison with advanced ocular lesions (scores 2 and 3), whereas only four animals represented the earliest stage of the disease (score 1) and thirteen animals showed no ocular lesions. Consequently, the bacterial profile observed in advanced cases may not fully reflect the microorganisms involved in the initiation of IKC, as the ocular microbiota is known to change during disease progression and secondary colonization may occur [22]. Future studies should therefore include a larger number of animals, particularly those examined at the earliest stages of clinical disease, when primary etiological agents are most likely to be detected before substantial changes in the ocular microbiota occur.

The histopathological findings observed in the present study were consistent with the spectrum of ocular lesions previously described in European bison with IKC, including epithelial alterations,

mononuclear inflammatory infiltrates, and lymphoid follicle formation in the conjunctiva, as well as epithelial changes, stromal edema and, in more advanced cases, neovascularization and connective tissue proliferation in the cornea [13]. Most of the histopathological lesions observed in the present study were mild, and their severity was not significantly associated with the detection of the examined bacteria. A similar lack of association between the presence of a potential infectious agent and lesion severity was previously observed in relation to *Thelazia* infection in European bison [13]. Histopathological findings reported in other cervids further highlight the variable nature of IKC, ranging from corneal erosion and stromal inflammation in red deer [23] to severe necrotizing and vascular lesions associated with CvHV2 infection in reindeer [16, 24]. Furthermore, experimental inoculation of reindeer with *M. bovoculi* alone did not result in detectable histopathological lesions, further suggesting that the presence of this bacterium does not necessarily result in ocular pathology [24]. Overall, these findings support the multifactorial nature of IKC and suggest that the presence of individual bacterial pathogens alone may not determine the severity of ocular lesions. Interestingly, bacterial detection was significantly more frequent in younger European bison. Similar age-related patterns have been described in domestic and wild ruminants, in which young animals are more susceptible to colonization by ocular pathogens, although colonization does not necessarily lead to clinical disease [10, 11]. Significantly lower body weight of European bison positive for analyzed microorganisms was most likely simply a consequence of their younger age.

Conclusion

The recent outbreak of IKC in European bison in Poland has been comprehensively investigated in a series of our previous studies addressing its epidemiology, risk factors, pathology, parasitology and ocular microbiota [12–15]. These studies demonstrated that disease severity was associated with mountainous habitats and the winter season [13], whereas infection with *Thelazia* spp., despite its relatively high prevalence, could not fully explain the occurrence of ocular disease [12, 14]. Likewise, characterization of the ocular surface microbiota revealed that no single bacterial species appeared to represent the primary etiological agent of IKC, indicating that the disease is likely multifactorial [12, 15]. The present study further supports this conclusion, as neither *Moraxella bovoculi*, *Mycoplasma* spp. nor *Chlamydia* spp. showed a significant association with the occurrence or severity of ocular lesions.

Taken together, the present findings and our previous investigations indicate that IKC in European bison results from complex interactions among opportunistic and primary pathogens, parasitic infections, environmental conditions and host susceptibility rather than from a single infectious agent. Future studies should therefore identify *Mycoplasma* spp. and *Chlamydia* spp. isolates to the species level and perform molecular and phylogenetic analyses to clarify their epidemiological significance and potential role in the development of ocular disease. In addition, investigations involving larger numbers of European bison, particularly animals examined at the earliest stages of clinical disease, are needed to improve the likelihood of identifying the primary etiological agents before substantial alterations of the ocular microbiota and secondary bacterial colonization occur.

Methods

Sampling of European bison

Between 2023 and 2025, 55 European bison were culled under permits issued by the General Directorate for Environmental Protection in Poland and implemented by the Regional Directorates of the State Forests. The culling was conducted as part of the official population management program. Individual animals were selected based on apparent health problems, including traumatic injuries, infectious diseases, and ocular abnormalities.

Animals were humanely shot by professional hunters in accordance with animal welfare and safety regulations, under the supervision and with the assistance of qualified veterinarians. For each individual, data regarding sex, age, geographical origin (Bieszczady Mountains, north-eastern Poland, south-western Poland), genetic line (Lowland or Lowland-Caucasian genetic line), and date of sampling were recorded. Vegetation season was considered to last from May to October and winter season from November to April.

Conjunctival swab samples were collected aseptically from the conjunctival sac of both eyes of each animal, irrespective of the presence of ocular lesions, using separate Thermo Scientific™ Sterilin™ Universal Transport Medium (UTM) swabs suitable for the detection of *Chlamydia* spp., *Mycoplasma* spp., and viral pathogens. One swab was collected from each eye. Following collection, the samples were immediately frozen and stored at – 80°C until further molecular analyses.

Molecular detection of bacteria using real-time PCR

DNA was extracted from conjunctival swab samples collected from 41 European bison using the Syngen DNA Mini Kit (Syngen Biotech, Wrocław, Poland) according to the manufacturer's instructions. For the remaining 14 animals, DNA was isolated from conjunctival sac tissue using [Sherlock AX, A&A Biotechnology, Gdańsk, Poland] according to the manufacturer's protocol. Following extraction, DNA samples were stored at – 20°C until analysis.

Real-time PCR assays were performed for the detection of *M. bovis*, *M. bovoculi*, *Chlamydia* spp., and *Mycoplasma* spp. cluster I (non-mycoides cluster) using commercially available kits (GPS™, Genetic Analysis Strategies S.L., Orihuela, Alicante, Spain) according to the manufacturer's protocols. Amplification reactions were carried out using a [QuantStudio 5, ThermoFisher Scientific, Waltham, USA]. An internal amplification control provided with the kits was included in each reaction to monitor PCR performance. Positive and negative controls supplied with the kits were included in each run to ensure assay reliability.

Samples were analyzed individually. Results were interpreted according to the manufacturer's recommendations, and samples with cycle threshold (Ct) values < 40 were considered positive.

Gross and histopathological examination of the eyes

Gross ocular lesions were evaluated during post-mortem examination by an experienced veterinarian, as previously described by Filip-Hutsch et al. [13]. The severity of ocular lesions was graded on a four-point scale (0–3), where score 0 represented clinically normal eyes; score 1, increased lacrimation and/or mild conjunctivitis (mild lesions); score 2, conjunctivitis and keratitis accompanied by corneal ulceration, purulent ocular inflammation, or fibrin deposition (moderate lesions); and score 3, severe lesions associated with deformation of the eyeball.

Following gross examination, eyes were fixed in 70% ethanol. The adequacy of fixation was assessed in the laboratory, and insufficiently fixed samples were transferred to 10% neutral buffered formalin for an additional 72 h. After fixation, a complete cross-section through the anterior segment of the eye together with the conjunctival sac was performed. Tissue samples were macroscopically evaluated, dehydrated through graded ethanol and xylene, embedded in paraffin wax, sectioned at 3–4 μm , and stained with hematoxylin and eosin (H&E).

Microscopic examinations were performed using an Axiolab 5 optical microscope equipped with an Axiocam 208 color camera (Carl Zeiss Microscopy GmbH, Jena, Germany) at 5 $\times$, 10 $\times$, and 40 $\times$ magnifications. Representative lesions were documented photographically. Histological sections were examined for the presence and distribution of inflammatory lesions and other pathological changes.

Statistical analysis

Numerical variables were examined for normality of distribution using the normal probability Q-Q plots and Shapiro-Wilk test. As the assumption of normality was violated for age ($p = 0.002$) and body weight ($p = 0.002$), the numerical variables were summarized as the median, interquartile range (IQR), and range and compared between groups using Wilcoxon's signed rank test (2 paired groups) or the Mann-Whitney U test (2 unpaired groups). Correlations between numerical variables were examined using Spearman's rank correlation coefficient (R_s). The most optimal cut-off value for numerical variables was determined using the method based on maximization of Youden's index [25]. Categorical variables were summarized as the frequencies (n) and proportions (%), and 95% confidence intervals (CI 95%) were calculated according to Wilson's score method [26]. Proportions were compared between paired groups using McNemar's test with Yates' continuity correction (2 groups) or Cochran's Q test (> 2 groups). Categorical variables were compared between unpaired groups by the maximum likelihood G test (bison-level analyses) or the mixed-effect logistic regression model with a bison fitted as a random effect (eye-level analyses) to control for the dependence of observations. Strength of associations was expressed as odds ratios (OR). All tests were two-sided and the significance level (α) was set at 0.05. Statistical analysis was carried out in TIBCO Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA) and IBM SPSS Statistics 31 (IBM Corporation, Armonk, NY).

Abbreviations

IKC
infectious keratoconjunctivitis

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' contributions

KFH: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. MC: Formal analysis, Statistical analysis, Data curation, Data validation, Visualization, Writing – review & editing. AR: Histopathological examination, Investigation, Visualization, Writing – review & editing. RS: Histopathological examination, Investigation, Writing – review & editing. MK: Molecular analyses, Investigation. WB: Investigation, Sample collection. WO and KA: Supervision, Project administration, Writing – review & editing. All authors read and approved the final manuscript.

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Figures

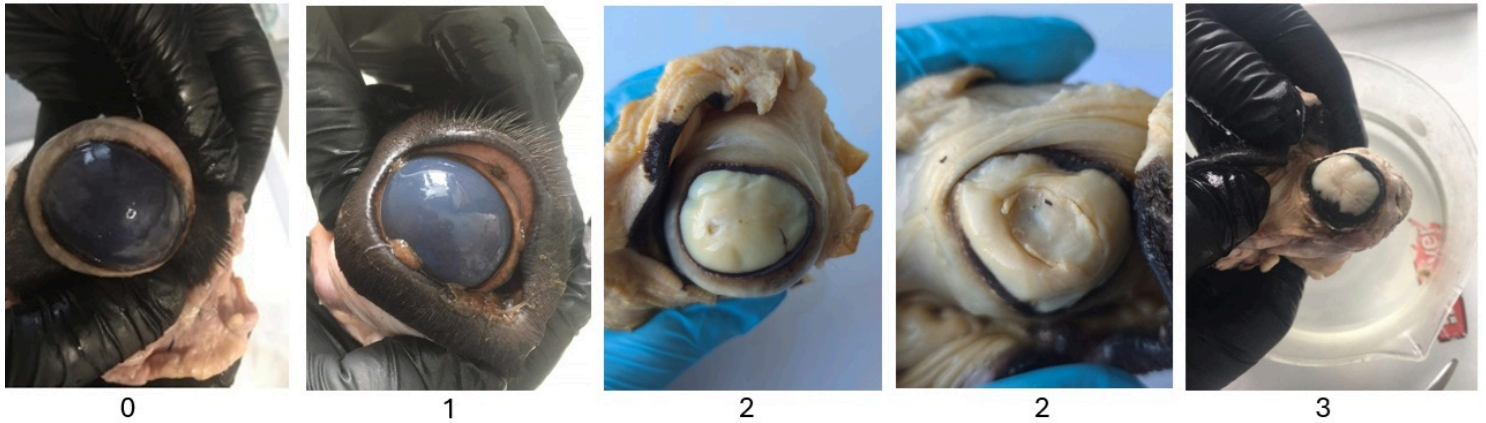


Figure 1

Representative ocular lesions in European bison (*Bison bonasus*) according to the severity score (0–3). Score 0 represents clinically normal eyes; score 1, mild lesions characterized by increased lacrimation and/or mild conjunctivitis; score 2, moderate lesions characterized by corneal opacity with focal ulceration (left image) or more advanced corneal ulceration (right image); and score 3, severe lesions associated with deformation of the eyeball.

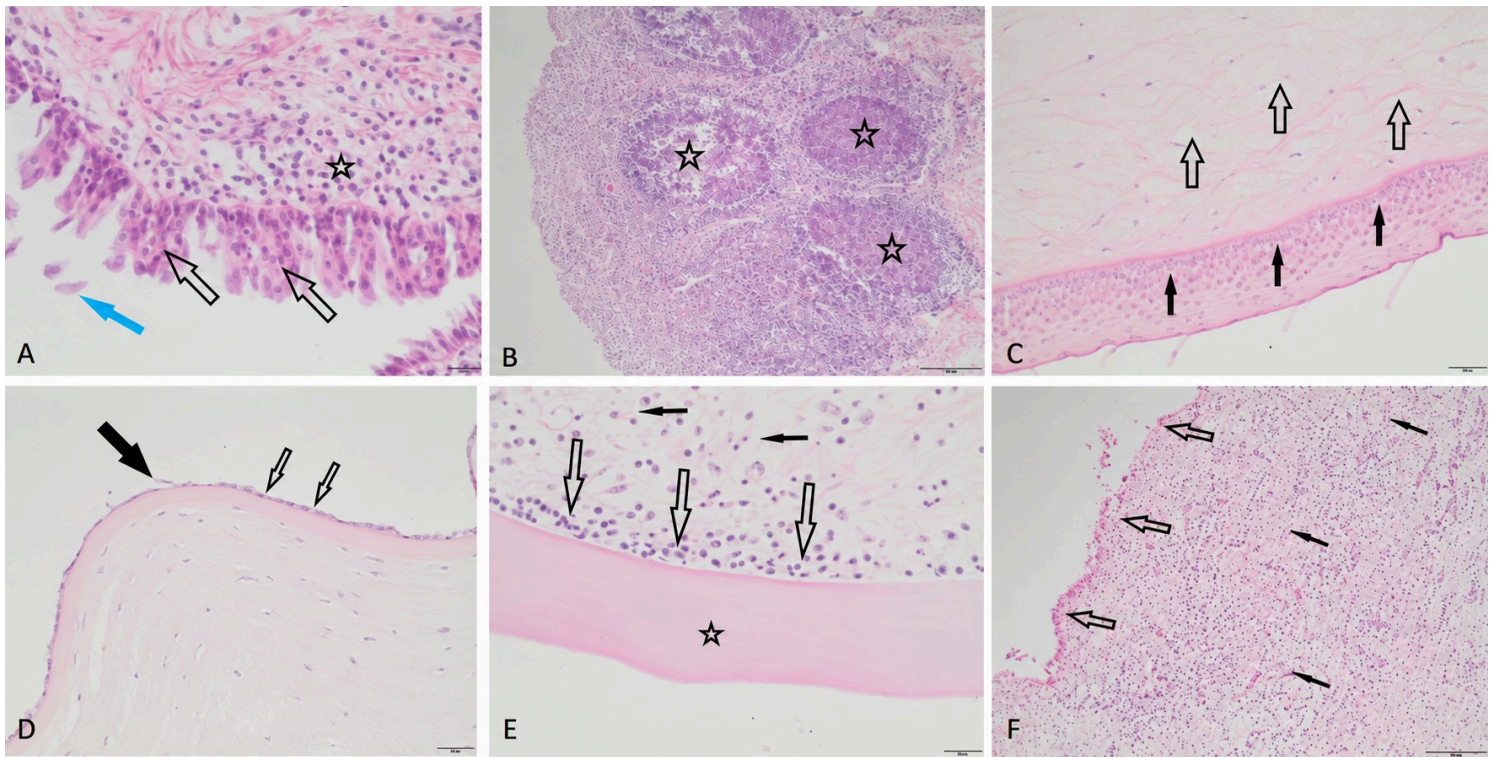


Figure 2

Representative histopathological lesions in the conjunctiva and cornea of European bison.

(A) Conjunctiva, 200×: epithelial cell hyperplasia (white arrows), epithelial vacuolation and exfoliation (blue arrows), subepithelial edema, and mononuclear inflammatory infiltrates in the lamina propria (star). (B) Conjunctiva, 400×: marked mononuclear inflammatory infiltrates in the lamina propria with lymphoid

follicle formation (stars). (C) Corneal epithelium and stroma, 100×: vacuolation of basal epithelial cells (black arrows) and stromal edema (white arrows). (D) Corneal endothelium, 100×: cellular vacuolation (white arrows) and cell exfoliation (black arrows). (E) Corneal stroma, 100×: sub-Descemet inflammatory infiltrates with a high proportion of plasma cells (white arrows), stromal edema (black arrows), and thickened Descemet's membrane (star). (F) Corneal epithelium and stroma, 40×: epithelial cell exfoliation and corneal ulceration (white arrows), diffuse and perivascular inflammatory infiltrates, stromal edema, and vascularization (black arrows).

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