Title: A Survey on Causative Agents of Infectious Bovine Keratoconjunctivitis in Industrial Dairy Farms in Alborz Province, Iran

Running title: Infectious Bovine Keratoconjunctivitis in Industrial Dairy Farms in Iran

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Abstract

Introduction: Infectious bovine keratoconjunctivitis is one of the most common eye diseases of cows worldwide, and is one of the diseases of dairy farms that can lead to significant economic problems. Along with *Moraxella bovis*, the main cause of keratoconjunctivitis, infectious bovine rhinotracheitis virus, and *Mycoplasma* species are probably the most important risk factors for keratoconjunctivitis. This study aimed to investigate the causes of keratoconjunctivitis on dairy farms in Alborz province, Iran. **Material and methods:** Between January 2022 and December 2022, a total of 40 animals with clinical symptoms of infectious keratoconjunctivitis (conjunctivitis and cornea, hyperemia, discharge, blepharospasm, and photophobia) were selected from 8 industrial dairy farms in Alborz province, and five swab samples were taken from each animal's eyes. For *Moraxella bovis* cultivation, swabs were cultured on blood agar at the farm. To detect IBR and mycoplasma, swabs were sent to the laboratory on ice for a PCR test. **Results:** Among 40 samples taken from cows with keratoconjunctivitis in 8 dairy farms in Alborz province, *Moraxella bovis* were isolated from 30% (12/40), BHV-1 virus 5% (2/40), *Mycoplasma* 25% (10/40), and *Mycoplasma* 25% (10/40) of the animals. In 40% of cases, despite clinical symptoms, no organism was isolated. **Conclusion:** BHV-1 virus, *Moraxella bovis*, and *Mycoplasma* play a major role in the occurrence of Keratoconjunctivitis. These findings emphasize the importance of accurate diagnostic testing to identify the pathogens involved and of implementing comprehensive management and hygiene protocols to effectively control this disease on industrial dairy farms.

Keywords: Bacterial agents, Dairy farms, Infectious bovine keratoconjunctivitis, Iran, Viral agents

1. Introduction

Infectious bovine keratoconjunctivitis, commonly known as "pink eye" of cows, is one of the most important and common eye diseases in cattle worldwide [1]. This disease has long interested veterinarians and dairy farmers due to significant economic losses from weight loss, reduced milk production, treatment costs, and, in some cases, blindness in cattle. This condition, especially on industrial dairy farms with high population density, can spread rapidly and become an epidemic, underscoring the need for its accurate recognition and optimal management [2, 3].

The main agent of bovine keratoconjunctivitis is the known bacterium *Moraxella bovis*. This bacterium produces toxins that cause characteristic corneal and conjunctival lesions. *M. bovis* possesses several pathogenic factors, including Q-type pili that help the bacteria attach to ocular cells and hemolysins that cause cell destruction. Understanding these pathogenic mechanisms is crucial for developing effective control and treatment strategies [4].

However, growing scientific evidence over the past few decades suggests that the etiology of infectious bovine keratoconjunctivitis is far more complex than a single pathogen can account for. Several studies have confirmed the role of other microbial agents, including *Moraxella bovoculi*, *Mycoplasma bovoculi*, and *Mycoplasma bovis*, in the onset and exacerbation of bovine keratoconjunctivitis [5]. These pathogens may not act alone; in most cases, a polymicrobial infection and synergistic cooperation among them lead to the development of the clinical form of bovine keratoconjunctivitis [6].

Non-microbial predisposing factors also play a crucial role in the epidemiology of bovine keratoconjunctivitis. Intense sunlight, dust, wind, flies as mechanical vectors, nutritional deficiencies (especially vitamin A), and stresses from transport or dietary changes can all weaken ocular defenses and pave the way for pathogen establishment and multiplication. Therefore, any intervention program must also carefully address these risk factors [7].

Globally, infectious bovine keratoconjunctivitis has been reported as a health and economic problem in all countries with a cattle industry, from North America and Europe to Australia and Asia [8, 9]. The outbreak of bovine keratoconjunctivitis negatively affects not only the economy of smallholder farmers but also the broader dairy supply chain [10]. In today's world, where food security and animal health are top priorities, controlling infectious diseases such as "pink eye" is a fundamental step towards sustainable, cost-effective livestock product production.

In Iran, with the increasing growth of the industrial livestock industry and the focus on increasing milk production, the health of dairy herds is of utmost importance [11, 12]. Alborz province, as one of the important hubs of dairy cattle breeding in the vicinity of the metropolis of Tehran, has a special place in the supply of dairy products. However, comprehensive, up-to-date information on the contribution and distribution of the various bacterial agents involved in infectious bovine keratoconjunctivitis on industrial dairy farms in this province is unavailable. The lack of such data has made it challenging to plan targeted, efficient control strategies.

Despite the economic impact of IBK on industrial dairy farms, knowledge gaps remain regarding its causative agents in many regions of Iran, including Alborz Province. Previous studies have predominantly focused on clinical diagnosis or single-agent identification, overlooking the potential role of co-infections or emerging pathogens such as *Moraxella bovoculi* and *Mycoplasma* spp. Furthermore, there is limited data on the seasonality and farm management factors that influence the prevalence of specific IBK pathogens in semi-arid climates such as Alborz. Therefore, the aim of this study is to determine the prevalence and distribution of bacterial agents responsible for IBK, including *Moraxella bovis*, *Moraxella bovoculi*, and other opportunistic pathogens, on industrial dairy farms across Alborz Province, and to identify the associated risk factors for disease occurrence.

2. Methods and materials

2.1. Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed, and we confirm that the study complied with the ARRIVE guidelines. The Animal Welfare Committee at Islamic Azad University has approved the study procedure. All the authors have checked Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and falsification, double publication and submission, and redundancy).

2.2. Study design and sampling

Between January 2022 and December 2022, a total of 40 animals with clinical symptoms of infectious keratoconjunctivitis (conjunctivitis and cornea, hyperemia, discharge, blepharospasm, and photophobia) were selected from 8 industrial dairy farms in Alborz province, and five swab samples were taken from each animal's eyes. In cattle with both eyes affected, samples were collected as a pool from both eyes. For *Moraxella bovis* cultivation, swabs were cultured on blood agar at the farm. To detect IBR and mycoplasma, swabs were sent to the laboratory on ice for a PCR test.

2.3.Polymerase chain reaction

First, DNA was extracted according to the kit instructions (PCR Pure, Roche). Then, PCR was performed with specific primers for IBR and Mycoplasma. For PCR, all five samples were pooled together (POOL). If the *Mycoplasma* genus was positive, species determination was performed with specific primers. To observe the PCR results, we loaded the samples onto a 1.5% gel. Next, using a UV-Illuminator device, the DNA bands were visualized (Table 1).

Table 1. PCR protocol: reagents, cycling steps, and primers

Category	**Specific Name / Step**	**Details**	**Amount / Temp / Time / Sequence**	**Expected Band**
Reaction Mix	ddH_2O		> 5.50 µL	
	Buffer		12.50 µL	
	Forward primer		> 1.00 µL	
	Reverse primer		> 1.00 µL	
	Template		> 5.00 µL	
	Total volume		25 µL	
	Initial Denaturation	Temperature (°C)	95	
		Time	15 min	
		No. of Cycles	1	
	Denaturation	Temperature (°C)	94	
		Time	30 sec	
		No. of Cycles	35	

	Annealing	Temperature (°C)	60	
		Time	90 sec	
		No. of Cycles	35	
Cycling Steps	Extension	Temperature (°C)	72	
		Time	180 sec	
		No. of Cycles	35	
	Final Extension	Temperature (°C)	72	
		Time	10 min	
		No. of Cycles	1	
	Hold	Temperature (°C)	4	
		Time	unlimited	
		No. of Cycles	unlimited	
	IBR-f	Sequence	CTGCTGTTCGTAGCCCACAACG	173 bp
	IBR-r	Sequence	TGTGACTTGGTGCCCATGTCGC	
	Mycoplasma spp-f	Sequence	GAAGAWATGCCWTATTTAGAAGATGG	319 bp
Primers	*Mycoplasma* spp-r	Sequence	CCRTTTTGACTYTTWCCACCMAGTGGTTGTTG	
	Mycoplasma bovis-f	Sequence	AAGGTACACCAGCTAACCCAG	300 bp
	Mycoplasma bovis-r	Sequence	AATGAAGCTACTGATCCAAG	

2.4.Antibiogram test

If *Moraxella* bacteria were isolated, a sensitivity test was performed using a standard method. Bacterial isolates from the samples, tested by culture, are assessed for susceptibility using standard antibiogram methods. In this way, soy tryptophan medium is used for 30 minutes until a standard turbidity of 0.5 McFarlane is reached. After that, Mueller-Hinton agar medium is used to create susceptibility discs. After that, Mueller-Hinton agar medium is used to create susceptibility discs. The discs are placed 2 cm apart and incubated for 24 hours at 37°C.

3. Results

Among 40 samples taken from cows with keratoconjunctivitis in 8 dairy farms in Alborz province, *Moraxella bovis* were isolated from 30% (12/40), BHV-1 virus 5% (2/40), *Mycoplasma* 25% (10/40), and *Mycoplasma* 25% (10/40) of the animals. In 40% of cases, despite clinical symptoms, no organism was isolated. In addition, no corneal ulcers were observed in the sampled animals. Also, positive cases of *Moraxella bovis* were antibiogram, and the results of sensitivity tests on isolated *Moraxella* species showed that the organism was susceptible to penicillin, ceftiofor, fluorophenicol, lincospectin, ampicillin, and amikacin, and to enrofloxacin, sulfamethoxazole + trimethoprim, tetracycline, and amotocillin. It is sensitive to gentamicin, streptomycin, neomycin, cobactan, cleistine, and erythromycin (Figure 1).

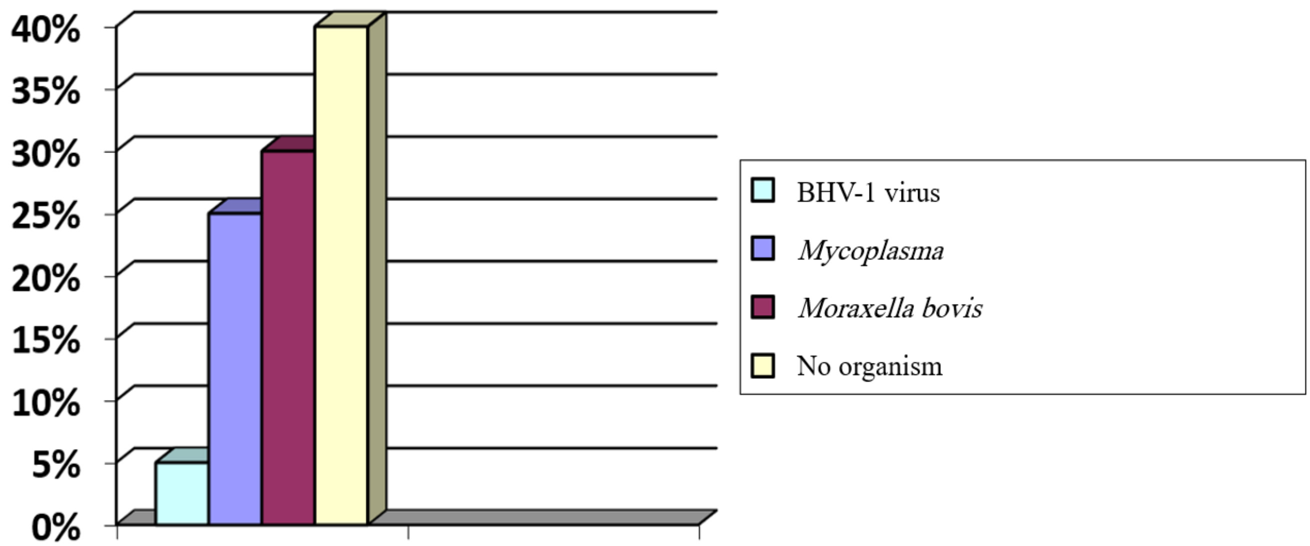

Figure 1. Frequency of identified causative agents among all samples taken from cows with keratoconjunctivitis.

4. Discussion

Infectious bovine keratoconjunctivitis is one of the most common eye diseases of cows worldwide and is among the diseases on dairy farms that can cause significant economic problems [9, 13, 14]. Along with *Moraxella bovis*, the leading cause of keratoconjunctivitis, infectious bovine rhinotracheitis virus, and *Mycoplasma* species are probably the most important risk factors for keratoconjunctivitis. This study aimed to investigate the causes of keratoconjunctivitis on dairy farms in Alborz province, Iran. Among 40 samples taken from cows with keratoconjunctivitis in 8 dairy farms in Alborz province, *Moraxella bovis* were isolated from 30% (12/40), BHV-1 virus 5% (2/40), *Mycoplasma* 25% (10/40), and *Mycoplasma* 25% (10/40) of the animals. In 40% of cases, despite clinical symptoms, no organism was isolated, suggesting that environmental factors such as dust, plant pollen, sunlight, and mechanical irritation can cause keratoconjunctivitis non-infectiously.

In a comprehensive international review conducted by researchers across different regions of the world, the epidemiology and risk factors associated with infectious bovine keratoconjunctivitis, also known as "pink eye," have been studied. A recent study by Maartens et al. (2025) conducted in dairy herds in the Mediterranean climate zone of South Africa reported a prevalence of 19.4% [15]. This study showed that *Moraxella bovoculi* was significantly associated with the disease, and factors such as breed (notably lower susceptibility of Jerseys), age (higher susceptibility of pre-weaned calves and heifers), availability of shade, fly load, and introduction of new animals to the herd were identified as important risk factors. Another study by Mubeen et al. (2024) conducted in commercial herds in Pakistan reported an overall prevalence of 6.86% [16]. This study emphasized the importance of management factors such as poor cattle and farm hygiene, high stocking density, exposure to sunlight, the presence of flies, and the lack of fly control programs, and also found that foreign breeds such as Holstein-Friesians were more susceptible than local breeds. Another study by Karthik et al. (2018) was conducted on an organized farm in India, and for the first time in this country, *Moraxella bovoculi* was identified as the causative agent of IBK [17] emphasized the role of the house fly (*Musca domestica*) in the transmission of IBK and the success of treatment with appropriate antibiotics based on drug susceptibility testing. A previous study by Takele and Zerihun (2000) conducted in Ethiopia reported a prevalence of 4.46% in crossbred herds [18]. This study found that young animals and those with higher levels of exogenous blood were significantly more likely to be infected and identified *Moraxella bovis* as the primary agent (80% of cases), with hemolytic strains playing an important role in causing ocular lesions. Taken together, these studies demonstrate that IBK is a multifactorial disease influenced by the interaction of different pathogens (particularly *Moraxella* species), host factors (breed, age, immune status) and environmental-management factors (flies, dust, UV light, shade, hygiene and stocking density) and that its effective control requires an integrated approach including biological management, insect control, environmental improvement and possibly vaccination.

In the current study, positive cases of *Moraxella bovis* were tested for antibiogram, and the results of sensitivity tests on isolated *Moraxella* species showed that the organism was susceptible to penicillin, ceftiofor, fluorophenicol, lincospectin, ampicillin, and amikacin, and to enrofloxacin, sulfamethoxazole + trimethoprim, tetracycline, and amotocillin. It is sensitive to gentamicin, streptomycin, neomycin, cobactan, cleistine, and erythromycin.

Conducting this study to identify the causative agents of infectious bovine keratoconjunctivitis on industrial dairy farms in Alborz province faced several challenges, the most important of which was the difficulty of collecting high-quality samples from affected animals due to the eyes' high sensitivity. Moreover, the working conditions in dairy environments, which are full of dust, ammonia, and other stressors, not only directly affect the animals' eye health but also make sterile sampling and proper transport of samples to the laboratory difficult. On the other hand, the accurate isolation and identification of the wide range of possible bacterial agents involved (including *Moraxella bovis*, *mycoplasmas*, and other opportunistic bacteria) and the determination of the main role of each in the pathogenesis of the disease required the use of advanced and expensive molecular techniques such as PCR and sequencing.

Given the findings of this study and the challenges ahead, it is suggested that future researchers worldwide should focus their studies on a more comprehensive understanding of pathogen-host-environment interactions in IBK disease, and by applying metagenomics and transcriptomics approaches, not only fully characterize the microbial community present in the eyes of infected and healthy cattle, but also explore the host immune response at the molecular level. Also, designing large-scale longitudinal studies to track disease dynamics across seasons and under different management regimes can identify key risk factors and sensitive points for preventive interventions. The development and evaluation of effective multipotent vaccines targeting multiple species and strains of *Moraxella bovis*, as well as other important secondary pathogens, should be a priority for future research. In addition, investing in the development of rapid, inexpensive, field-based diagnostic methods that can distinguish between primary and secondary pathogens can significantly contribute to timely management and reduce inappropriate antibiotic use. Finally, promoting international collaboration and forming research consortia to share epidemiological data, bacterial strains, and genetic resources will accelerate global understanding of this disease and the development of practical solutions to control it.

Conclusion

The results of this study showed that bacteria such as BHV-1 virus, *Moraxella bovis,* and *Mycoplasma* play a major role in the occurrence of Keratoconjunctivitis. These findings emphasize the importance of accurate diagnostic testing to identify the pathogens involved and of implementing comprehensive management and hygiene protocols to effectively control this disease on industrial dairy farms.

Declarations and statements

Funding

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Conflict of interest

The authors declare no conflict of interest.

Data availability

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

Ethical approval

The Ethics Committee of the Faculty of Medicine, Islamic Azad University, Karaj, Iran, approved this research.

Author contribution

Conceptualization: [A.H.], …; Methodology: [A.H., A.R.S., F.M.], …; Formal analysis and investigation: [A.H., A.R.S., F.M.], …; Writing - original draft preparation: [A.H., A.R.S., F.M.]; Writing - review and editing: [A.H., A.R.S., F.M.], …; Funding acquisition: [No funding], …; Supervision: [A.H.]. All authors checked and approved the final version of the manuscript for publication in the present journal.

Consent to participate

Not applicable

Consent for publication

Not applicable

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Declaration of the use of generative AI

Not used for any purposes

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