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< Case Report >

Hematological and microbial analysis on a Holstein heifer with infectious bovine keratoconjunctivitis

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Abstract

Infectious bovine keratoconjunctivitis (IBK) is the most common ocular disease in cattle, manifesting as corneal opacity, corneal ulcerations and potentially vision loss. The present report describes a 10-month-old Holstein Friesian heifer with IBK treated by systemic tulathromycin, and subconjunctival injection of penicillin and dexamethasone. We investigated changes in the hematological indices and microorganisms related to IBK after treatment. Neutrophils and monocytes decreased during recovery, so it was assumed that these two types of white cells are associated with IBK. *Moraxella bovoculi* was cleared in the eye, nasal cavity, and oral cavity after treatment. The distribution of *M. bovoculi* before treatment indicated that a combined systemic and subconjunctival treatment was necessary. The lesioned eye was found to be overwhelmed by *Mycoplasma bovoculi*, while pathogen abundance was reduced in the nasal cavity and oral cavities. These results suggest that antibiotic treatment can alter the composition and relative abundance of microorganisms.

Key words : Infectious bovine keratoconjunctivitis, Holstein heifer, *Moraxella bovoculi*

INTRODUCTION

The most common ocular disease in cattle is infectious bovine keratoconjunctivitis (IBK or "pinkeye") which affects all breeds but has been reported most frequently in lighter-colored breeds, such as Hereford, Hereford-crossbred cattle, Jerseys and Friesian cattle (Wilcox, 1968; Frisch, 1975; Ward and Nielson, 1979; Webber and Selby, 1981; Snowder et al, 2005; Angelos, 2015). IBK mainly occurs between spring and fall, peaking in summer. However, hay feeding contributes to the incidence of IBK from late fall to early spring, such that IBK can break out all year round (Boileau et al, 2015; Snowder et al, 2005).

Moraxella bovis and *Moraxella bovoculi* are involved in IBK; both of these microorganisms colonize the eyes of cattle, although healthy cattle may also have both of these microorganisms in their eyes (Angelos, 2015; Schnee et al, 2015). Cattle that are exposed to ultraviolet radiation, flies, mycoplasma spp. infection, IBR virus infection and foreign bodies are more likely to develop IBK (Hughes et al, 1965; Steve and Lilly, 1965; Pugh Jr et al, 1970; Pugh et al, 1976; Glass Jr et al, 1982; Rosenbusch, 1983; Arends et al, 1984; Lepper and Barton, 1987; George et al, 1988; Angelos, 2015). Cattle with clinical signs of IBK may show photophobia, lacrimation, corneal opacity (edema), and corneal ulcerations and can experience vision loss (Alexander, 2010). IBK also exerts detrimental effects on the economy, as well as on animal welfare. IBK is difficult to be prevented because neither commercially available nor autogenous vaccines against *M. bovis* and *M. bovoculi*. are effective (Funk et al, 2009;

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Cullen et al, 2016).

Changes in hematological indices and the relative abundance of microorganisms associated with IBK have never been reported in, Korea or elsewhere. The present report describes hematological changes in cases where IBK pathogens are detected, and how to treat IBK.

CASE REPORT

A 10-month-old female Holstein Friesian heifer presented with IBK on May 11, 2018. From a distance, we observed blepharospasm, lacrimation, and photophobia in the right eye. she was restrained in a stanchion for a closer examination. The right eye exhibited epiphora and corneal opacity (edema). The corneal lesion had circumferential vascularization (Fig. 1). Among the many pharmacological treatment options, tulathromycin and a subconjunctival injection of penicillin and dexamethasone were administered. Xylazine (46.64 mg; Rompun®) was administered intravenously to induce deep sedation before treatment. Three medications were applied in combination, three times over 9 days (4-day interval). Penicillin G benzathine hydrate (100,000 IU), penicillin G procaine (150,000 IU of DS Long Acting PPS®), and dexamethasone 21-disodium phosphate (1 mg; Dexorone®) were administered by subconjunctival injection. Tulathromycin (500 mg; Draxxin®) was injected subcutaneously. The eye lesions steadily improved, and opacity decreased day by day. However, it took about 2 months for the opacity to completely disappear.

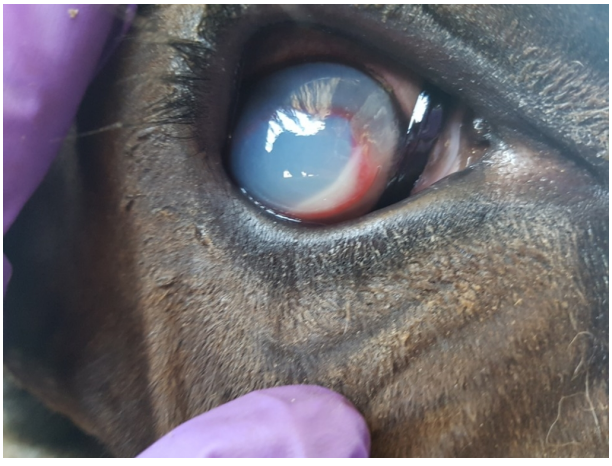

Fig. 1. The right eye of a Holstein heifer with infectious bovine keratoconjunctivitis (IBK). The corneal opacity (edema) is surrounded by vascular ingrowth.

Blood and swab samples were collected to determine changes in the hematological and microbiological parameters after treatment. Peripheral blood samples were obtained through jugular vein. The hematological analysis was performed with an automated hematology analyzer (Procyte Dx® Hematology Analyzer; IDEXX Laboratories, INC., Westbrook, MN, USA) to evaluate the immunological changes. Swabs were used on the surface of the eye, and in the nasal cavity, mouth, and rectum to identify which microorganisms caused IBK, where they occurred in the body, how the microbiota was different from that of a clinically normal heifer (control) randomly selected from the same barn, and how the microorganisms changed after treatment. The control heifer was born a month earlier than the patient heifer. They kept being raised at the same barn with the same animal husbandry techniques. Swab samples were analyzed by next-generation metagenomics sequencing (Macrogen Inc., Seoul, Korea). DNA was extracted using a DNeasyPowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted DNA was quantified using Quant-IT PicoGreen (Invitrogen). The sequencing library is prepared according to the illumine 16S Metagenomic Sequencing Library protocols to amplify the V3 and V4 region. The input gDNA 2 ng was PCR amplified with 5x reaction buffer, 1 mM of dNTP mix, 500 nM each of the universal F/R PCR primer, and Herculase II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA). The cycle condition for 1st PCR was 3 min at 95°C for heat activation, and 25 cycles of 30 secs at 95°C, 30 secs at 55°C and 30 secs at 72°C, followed by a 5-min final extension at 72°C. The universal primer pair with Illumina adapter overhang sequences used for the first amplifications were as follows: V3-F: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3', V4-R: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'. The 1st PCR product was purified with AMPure beads (Agencourt Bioscience, Beverly, MA). Following purification, the 2 μL of 1st PCR product was PCR amplified for final library con-

struction containing the index using NexteraXT Indexed Primer. The cycle condition for 2nd PCR was same as the 1st PCR condition except for 10 cycles. The PCR product was purified with AMPure beads. The final purified product is then quantified using qPCR according to the qPCR Quantification Protocol Guide (KAPA Library Quantificatoin kits for IlluminaSequecing platforms) and qualified using the TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). The paired-end (2×300 bp) sequencing was performed by the Macrogen using the MiSeq™ platform (Illumina, San Diego, USA).

The peripheral white blood cell count was in the reference range despite the presence of IBK. The heifer had a leukocyte level 10.5 K/μL, neutrophil level of 2.72 K/μL, lymphocyte level of 5.6 K/μL, monocyte level of 0.66 K/μL, eosinophil level of 1.49 K/μL, and basophil level of 0.03 K/μL before treatment. The white blood cell count decreased after treatment by 0.77 K/μL. Neutrophil and Monocyte counts increased by 0.68 and 0.51 K/μL, respectively, whereas lymphocyte, eosinophil, and basophil counts decreased by 1.33, 0.6, and 0.03 K/μL, respectively (Fig. 2).

A few microorganisms related to IBK were detected in both the patient and control heifer, even though they were not discovered in the feces. *Mycoplasma bovoculi* and *Mycoplasma penetrans* were isolated from the same region. The oral cavity of the patient heifer contained *Moraxella bovoculi*. The percentage of *Moraxella bovoculi* in the patient heifer was about 82 and 3 times higher than in the control heifer in the eye and nasal cavity, respectively (Table 1). Once all of the clinical signs of IBK in the patient heifer had disappeared, including corneal opacity, we determined whether *Moraxella bovoculi* and *Mycoplasma penetrans* had been cleared. The lesioned eye was found to be overwhelmed by *Mycoplasma bovoculi*, while the microbe concentrations had decreased in other regions (Table 2). *Moraxella bovoculi* and *Mycoplasma* spp. were still present in the control heifer, but their proportions had changed (Table 3).

DISCUSSION

This case was presented in spring well-known for infectious bovine keratoconjunctivitis (IBK) to frequently occur (Snowder et al, 2005). Following that in spring,

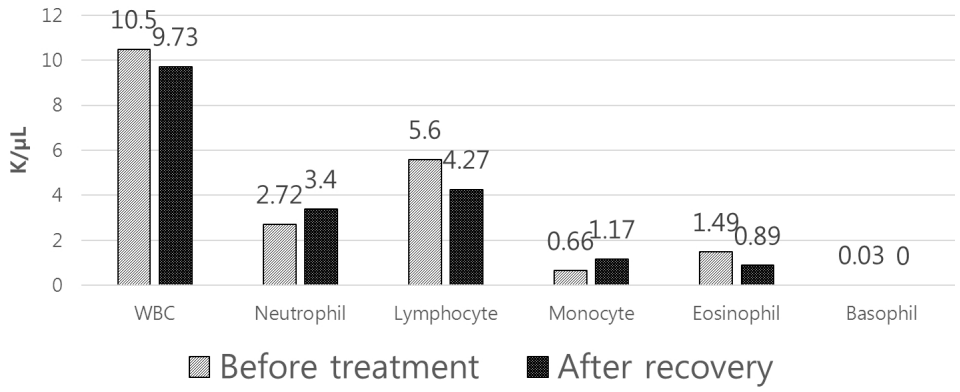

Fig. 2. Changes in the number of leukocytes after treatment. Neutrophil and monocyte counts increased, while lymphocyte, eosinophil, and basophil counts decreased.

Table 1. Relative abundance of microbes related to Infectious bovine keratoconjunctivitis (IBK) in a patient heifer and a clinically normal heifer (control)

	Patient heifer			Clinically normal heifer		
	Eye	Nasal cavity	Oral cavity	Eye	Nasal cavity	Oral cavity
Mycoplasma bovoculi (%)	71.98	1.22	0.04	52.54	0.05	0.03
Moraxella bovoculi (%)	4.10	0.23	0.08	0.05	0.07	0
Mycoplasma penetrans (%)	0.97	7.93	0	0.01	3.39	0

All microbial species known to be associated with IBK were found in the eyes and nasal cavity of both heifers.

Table 2. Changes in the relative abundance of microbes related to infectious bovine keratoconjunctivitis (IBK) after treatment

	Before treatment			After recovery		
	Eye	Nasal cavity	Oral cavity	Eye	Nasal cavity	Oral cavity
Mycoplasma bovoculi (%)	71.98	1.22	0.04	94.98	0.51	0
Moraxella bovoculi (%)	4.10	0.23	0.08	0	0	0
Mycoplasma penetrans (%)	0.97	7.93	0	0	0	0

The proportion of *Mycoplasma bovoculi* increased, while *Moraxella bovoculi* and *Mycoplasma penetrans* were eradicated.

Table 3. Relative abundance of microbes related to infectious bovine keratoconjunctivitis (IBK) in a patient heifer after treatment and a control heifer

	Patient heifer after recovery			Control heifer		
	Eye	Nasal cavity	Oral cavity	Eye	Nasal cavity	Oral cavity
Mycoplasma bovoculi (%)	94.98	0.51	0	11.74	0.13	0.03
Moraxella bovoculi (%)	0	0	0	0.01	0.02	0
Mycoplasma penetrans (%)	0	0	0	0.08	0.6	0

The treated eye of the patient heifer was dominated by *Mycoplasma bovoculi*. The control heifer had the same microbial complexes related to IBK before even though the relative abundance changed. The percentage of *Mycoplasma bovoculi* decreased.

ultraviolet radiation becomes stronger, flies thrive, and *Mycoplasma* spp. were also detected with metagenome sequencing (Hughes et al, 1965; Steve and Lilly, 1965; Pugh Jr and Hughes, 1968; Pugh et al, 1976; Glass et al, 1982; Rosenbusch, 1983; Arends et al, 1984; Lepper and Barton, 1987), It is difficult to determine what caused IBK in our heifer. This is the first case report in Korea describing changes of the peripheral white blood cell count, pathogenic microorganisms, and pathogen distribution in a Holstein heifer infected with IBK after treatment.

The total number of peripheral white blood cells was in the reference range both before and after treatment, even though the count decreased slightly. Some kinds of leukocytes increased in number, while others decreased. A case study reported lymphocytopenia in associated with IBK (Lasisi and Akinbobola, 2016). However, in the present case, lymphocytes were not involved in IBK; the number of lymphocytes in the normal range and decreased after treatment. Neutrophils recruited to the area of infection were damaged by the hemolysin produced by *M. bovis*. and both the neutrophils and corneal epithelial cells were lysed and killed by a cytotoxin encoded by a. repeats-in-toxin class operon produced by *M. bovoculi* (Kagonyera et al, 1989; Beard and Moore, 1994; Angelos et al, 2007). This may explain why the neutrophil count was decreased before, and increased after, treatment. The number of monocytes in peripheral blood changed, similar to neutrophils, and more monocytes were detected after than before treatment; this suggests that monocytes are associated with IBK, similar to neutrophils.

The composition of microorganisms related to IBK was similar between the control and patient heifer, even though the relative abundance was different, indicating that colonization by *Moraxella bovoculi* can occur in cattle at subclinical levels. Other intrinsic or extrinsic factors may also play a role in inducing *Moraxella bovoculi* to become pathogenic (Angelos, 2015). It is not clear why *Mycoplasma bovoculi* dominated the recovered eye. *Mycoplasma bovoculi* may be resistant to the antibiotics or newly settle after the flora in the lesion were destroyed in the present case. The microorganisms in the recovered eye were different from those in the control. Further study is needed to identify microorganisms in the eye lesion post-antibiotic treatment.

Many pharmacological treatments have been applied for IBK. In particular, antibiotics have been administered via intramuscular, subcutaneous, subconjunctival, and topical routes, and via combinations thereof. However, treatment may fail due in cases of severe corneal ulceration, or antibiotic resistance (Angelos, 2015). Given

that bacteria related to IBK are distributed throughout the eye, nasal cavity, and oral cavity, and the eye, nasal cavity, and oral cavity are connected, choosing only one treatment might lead to treatment failure. It is difficult to achieve a high drug concentration via systemic administration, and subconjunctival injection of antibiotics is insufficient to eliminate pathogens in the nasal and oral cavity (Maggs et al, 2013). Treating cattle with IBK by combined systemic and sunconjunctival administration of antibiotics may be the ideal approach.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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