Study of the usefulness of tear components in respiratory disease of Japanese Black Calves

Summary of Doctoral Thesis

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Summary

Calves have an immature immune system compared to adult cows and are more susceptible to various infectious diseases. According to the Livestock Mutual Aid Statistics, most injuries are from gastrointestinal disease, followed by respiratory disease. Symptoms of gastrointestinal disease include changes in stool properties and adhesion of diarrheal stool to the ridge, which are easy for the owner to visually detect. However, coughing, runny nose, etc., which are symptoms of respiratory diseases, are not inevitable, and often only become severe when the symptoms become clear. One of the factors involved in this is that there is no index of initial symptoms that the owner can easily grasp visually. Respiratory diseases in Japanese black calves are caused by viral infection preceded by indigenous bacteria existing in the nasal cavity invading the upper respiratory tract and lungs. In recent years, infection prevention measures such as vaccination and administration of first milk preparations have been taken, but many calves still suffer from respiratory diseases, and the economic loss to Japanese beef farmers is large. Since many calves have tears at the first presentation of Japanese black calves suffering from respiratory diseases, it is considered that there is a relationship between respiratory diseases and tear volume. In recent years, non-invasively collectable tear fluid has been actively studied as a biomarker for diseases not only in human medicine

but also in small animal and veterinary medicine. This study was conducted to investigate whether changes in lacrimal properties are useful as an index for detecting the acute inflammatory phase of calves suffering from respiratory diseases.

In the second chapter, the tear volume of calves diagnosed as clinically healthy was measured, the total protein concentration in tear fluid was measured, and the factors affecting the tear volume were examined. The amount of tears was measured according to Schirmer Tear Test (STT) method I, indicated as the STT I value. The STT I value of clinically healthy Japanese Black calves was 18.9±2.9 mm/min (n=263). The STT I value (20.6±3.8 mm/min) of cattle younger than 15 days (n=33) was significantly higher compared with those older than 60 days (n=45) (17.7±1.9 mm/min). Therefore, the statistical analyses of environmental variables were limited to cattle older than 15 days. There were no significant correlations between STT I values and the environmental temperature, humidity, and illuminance, or time of day. However, the STT I value was significantly positively correlated with the ammonia concentration in the barn. The total protein concentration in tears was 1.18±0.30 mg/ml (n=38) in clinically healthy Japanese Black cattle from 15 to 90 days of age. It was clarified that the factors affecting STT I value are the age after birth and the ammonia concentration in the barn.

In the third chapter, we examined whether changes in lacrimal properties are useful as an index for detecting the acute inflammatory phase of calves suffering from respiratory diseases. In section 1 of this chapter, the STT I values and protein concentrations in tears of calves with respiratory diseases were examined. The STT I value (22.2±3.0 mm/min, n=63) of calves in the acute inflammatory stage of respiratory disease was significantly increased compared with clinically healthy calves. Upper respiratory tract infection is said to increase tear production due to irritation and inflammation of the nasal mucosa, and upper respiratory tract infection is considered to be a factor in tear production in calves during the acute inflammatory phase of respiratory disease. In addition, the total protein concentration in the tear fluid of calves during the acute inflammatory stage of respiratory disease (1.85±0.47 mg/ml, n=63) was significantly higher compared with that of clinically healthy calves (1.15±0.31 mg/ml, n=62).

In section 2 of the third chapter, comparative identification analysis of protein components in tear fluid was performed using two-dimensional electrophoresis and liquid chromatography-mass spectrometry (LC-MS/MS). From the results of two-dimensional electrophoresis, 1,329 spots were detected in clinically healthy calves. Forty-nine calves in the acute inflammatory stage of respiratory disease have more than twice the number

of protein spots compared to clinically healthy calves, with the most variability around 25kDa. Identification analysis of these three spots using LC-MS/MS did not lead to identification, but it was suggested that they may be the L chain of immunoglobulin.

Therefore, in section 3 of the third chapter, the usefulness of tear fluid properties in respiratory diseases was examined by evaluating the immunoglobulin concentration in tear fluid, including digestive diseases typical of calves. IgA concentration in the tear fluid of the acute inflammatory stage of respiratory disease was significantly higher compared with clinically healthy calves and calves of gastrointestinal disease. This suggests that this is the main factor in the increase in the total protein concentration in the tear fluid. It was thought. It has been reported that in inflammatory diseases of the ocular mucosa such as acute bacterial conjunctivitis, palpebral conjunctivitis, and acute keratoconjunctivitis, IgA production is locally increased, so that the IgA concentration in tears increases but the IgG concentration does not change. In this study, the increase in tear fluid and IgA in tear fluid observed in calves with respiratory diseases was considered to be caused by irritation to the conjunctiva and cornea, which are the upper respiratory tract and ocular mucosa, caused by respiratory diseases.

In section 4 of the third chapter, we examined changes in tear properties during the acute inflammatory stage of respiratory disease. A follow-up study of calves found high tear

fluid IgA levels (0.99±0.08 mg/ml, n=7), despite being evaluated as clinically healthy calves at the time of tear collection. Within a few days, changes to conditions suggestive of the acute inflammatory stage of respiratory diseases such as fever and abnormal respiratory patterns such as urgency were observed. Therefore, when tears were collected over time for the two calves evaluated as clinically healthy, the high IgA concentration in the tears was found before the onset of external symptoms of respiratory disease. This suggested that changes in lacrimal properties may be useful as an index for detecting the acute inflammatory phase of respiratory diseases.

Until now, there has been no clear index for the acute inflammatory phase of respiratory disease, but it was suggested that observing the circumference of the eye to see if the tear volume is increasing may be useful as one of the indicators. This is considered to indicate the importance of observing the eye circumference not only for livestock farmers, whose number of cattle raised per household has been increasing in recent years, but also for all breeding Japanese beef farmers. In winter, if one cow suffers from respiratory disease, there is concern that the infection will spread to the entire herd. If it is possible to observe the area around the eyes and treat it in the early stages of respiratory illness during daily feeding management, reduce the number of treatment days, prevent the spread of infection, and reduce the number of illnesses and injuries caused by livestock mutual aid.

It is thought that this will lead to a reduction in infections, which in turn will contribute to better calf breeding, productivity improvement, and stable farm management.