別記様式第３号

学　位　論　文　要　旨

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題　　目　Studies on the development of a novel HACCP-based approach to the control of infectious diseases in a cattle farm.
（牛農場における感染症制御への HACCP に基づく新たなアプローチの開発に関する研究 ）

Neonatal calf diarrhea (NCD) is one of the major diseases in the cattle industry worldwide, causing substantial economic losses due to high morbidity, mortality, growth retardation and treatment costs, as well as serious long-term consequences such as delayed first calving. NCD is the most common cause of death in calves during their first 30 days of life, with a case fatality risk of approximately 5%. In Japan, the economic losses due to calf diarrhea are estimated at approximately one billion yen per year, according to the 2017 annual report from the Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF). The viral component of the microbiome (the virome) is receiving increasing attention for its role in NCD. The viral etiologies of diarrhea which cause considerable economic losses for bovine farms include bovine rotavirus A (RVA), bovine torovirus (BToV), bovine enterovirus (BEV) and bovine coronavirus (BCV); hence, those viruses were selected as candidate "viral pathogen indicators". Viral pathogen indicators within bovine farms in this study were tentatively defined as relatively highly prevalent viruses found at bovine farms in Japan.

Persistence of this significant problem in the field may be attributed to the multifactorial nature of calf diarrhea, including permutations of infectious diseases, lack of clear understanding of the disease ecology, poor environmental hygiene, and biased epidemiological data. Genetic diversity, continuous evolution, the emergence of pathogens, and/or the ubiquity of pathogens in the environment are factors that hinder effective control of the disease. MAFF has been introducing the "hazard analysis critical control point (HACCP) at livestock farm level: Farm HACCP Standard" certification standard for livestock farms in Japan since MAFF announced this certification in 2009 and the Farm HACCP Standard has been revised in 2022. The Farm HACCP system is a hygienic control system that manages hazards for the livestock industry in production environment. In this dissertation, the main objectives are to investigate crucial factors in

the implementation of a Farm HACCP system, which should be based on three major points to effectively control of calf diarrhea. First, a clear understanding of the characteristics of pathogens (e.g. prevalence in the field, and genetic evolution, etc.) is required. Second, to investigate the effectiveness of current vaccines and their ability to effectively confer protection against a variety of field pathogens. Finally, proper hygiene management is necessary for disease prevention and control. The findings of this study are summarized as follows:

In Chapter 1, RVA, BToV, BEV and BCV at a bovine farm in Ibaraki prefecture were monitored by one-step multiplex RT-PCR, with the aim of confirming the reduction of "viral pathogen indicators". After improvement of hygiene protocols on boots in a bovine operation (farm A) in Ibaraki, Japan in September 2017, mortality of calves less than 2 month-old and the detection of 4 viral pathogen indicators, became significantly low for one year. Subsequently, following one year improvement of hygiene protocols on boots these indicators and mortality were monitored and confirmed all were still low, except for the detection rate of bovine RVA in calves less than 3 weeks old. Molecular analysis using semi-nested multiplex RT-PCR of positive RVAs (n = 122) and sequencing of selected samples revealed the presence of G6, G8, G10, P[1], P[5] and P[11] genotypes and the prevalence of G and/or P combination and mixed infections. The most common combination of G and P types was G10P[11] (41.8%), followed by mixed infection with G6+G10P[5] (11.5%). Phylogenetic analysis of RVAs showed clustering with bovine and other animal-derived RVA strains, suggesting the possibility of multiple reassortant events with strains of bovine and others animal origins. Noteworthy as well is that vaccinated cattle may not be able to provide maternal immunity to their offspring against RVA infections, due to insufficient colostrum feeding. The results of the present study further highlight the importance of viral pathogen indicators surveillance in bovine populations, which may be useful to improving effective routine vaccination and hygiene practices on bovine farms. Besides, proper management of boots at calf sheds is important, perhaps even vital, for rearing hygiene measures at bovine farms so as to attain substantial decrease in the prevalence of pathogens.

In Chapter 2, RVAs were isolated from fecal samples containing different bovine RVA genotypes at farm A in Ibaraki, Japan and the isolated RVAs were serotyped by the serum neutralization assay and compared with RVA strains in a vaccine used at the farm. Antisera were prepared against the three isolates and the vaccine strains of bovine RVA. The results of cross-neutralization tests revealed that the RVA isolates from this farm differed somewhat in serotype. Collected plasma samples from calves for 6 weeks after colostrum ingestion showed that maternal antibodies acquired against all isolates gradually decreased, but antibodies toward one isolate not included in the vaccine strains increased by 6 weeks after the mentioned decreasing. These results suggest that vaccination with a

new vaccine containing the new RVA serotype is necessary to protect calves.

In Chapter 3, accurate evaluation methods for surface disinfection are hence required to effectively apply the disinfectants on the farms and improve biosecurity level. In the present study, RVA and BCoV were inactivated using food additive-grade calcium hydroxide ($FdCa(OH)_2$) solution, Quaternary ammonium compounds (QAC) and their mixture through suspension tests as the primary screening, and afterward via carrier tests using the dropping or dipping techniques as the secondary screenings. Viruses in the aqueous phase can be easily inactivated in the suspension tests, but once attached to the materials, they can become resistant to disinfectants, and require longer times to be inactivated. This highlights the importance of thorough cleaning with detergent before disinfection, and keeping elevated contact durations of proper disinfectants to reduce viral contamination and decrease infectious diseases incidence in farms. It was also reaffirmed that the suspension and carrier tests are necessary to evaluate disinfectants and thus determine their actual use. Particularly, the mixture of $FdCa(OH)_2$ and QAC was found to exhibit synergistic effects and broad-spectrum compared to their use alone, and is now recommended for use on livestock farms.

These findings suggest the importance of controlling prevalence of pathogens on bovine farms. It is possible to increase the level of biosecurity at bovine farms through the farm HACCP approach by proper vaccination, changing boots and clothing, the appropriate use of disinfectants, or limiting access to the farms. In such cases, the presence and detection of the prevalent indicator viruses will be decreased.

（備考）１　学位論文題目が日本語の場合は英訳を、英語の場合は和訳を（　　）内に記入すること。（英語の表記は，論文目録と同じ。）

２　日本語の場合は、２，０００字程度とする。

３　英語の場合は、１，２００語程度とする。

４　図表は、この要旨には記載しないこと。

５　枚数は１枚を超えても差し支えありません。