別記様式第３号

学　位　論　文　要　旨

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題　　目

Studies on epidemiology of viral pathogen indicators and biosecurity enhancement on pig farms

（豚農場内に蔓延する汚染指標病原体の疫学調査とバイオセキュリティの強化に関する研究）

To date, many preventive measures have been established and utilized to minimize the economic losses due to serious infectious diseases in the domestic livestock industry worldwide. Although these preventive measures have been utilized in the livestock industry, outbreaks of infectious viral diseases in livestock have caused significant economic losses worldwide, including Japan. Therefore, advanced preventive measures need to be established in addition to the conventional countermeasures.

On-farm disinfection is considered as one of the most effective actions to control infections on livestock farms. However, published evaluation methods of virucidal activities of disinfectants toward viruses on surfaces are not convenient and accurate, especially for the wiping method. Moreover, evaluation methods of efficacy of disinfectants toward microorganisms in the air are not published. Therefore, effective disinfection methods against viruses on surfaces and in the air need to be established and utilized to enhance the biosecurity at the farm level. Besides, for the improvement and assessment of the biosecurity levels in livestock farms, accurate information on pathogens on the farms needs to be shown. Since the reductions of indicator pathogens on the farms mean that biosecurity has been enhanced, data on ubiquitous pathogens surveillance is necessary to achieve the enhanced biosecurity on farms. Therefore, the main objective of this study is to enhance biosecurity in pig farms using effective disinfection strategies.

In Chapter 1, the efficacies of various disinfectants against infectious bronchitis virus (IBV) in aqueous phase and on plastic surfaces were tested. For the viruses on the surfaces, dropping and wiping decontamination techniques were established and performed. The disinfectants evaluated were 0.17% food additive glade calcium hydroxide ($FdCa(OH)_2$) solution, sodium hypochlorite at 500 or 1,000 parts per million (ppm) of total chlorine (NaClO-500 or NaClO-1,000, respectively), NaClO-500 supplemented in 0.17% $FdCa(OH)_2$ (Mixed-500) and quaternary ammonium compound (QAC) diluted 500-fold in water (QAC-500). In the suspension tests, all tested solutions inactivated the IBV to the undetectable level within 30 sec, even with the presence of 5 % fetal bovine serum (FBS). In the carrier test with the dropping technique, NaClO-1000 and QAC-500 could inactivate the virus with 0.5% FBS to undetectable level

within 1 min. $FdCa(OH)_2$ and Mixed-500 efficiently inactivated the virus (≥ 3 log_{10} reductions). With the wiping technique, all solutions, except NaClO-500, could inactivate the virus on a carrier to undetectable level in the wiping-sheets and wiped-carriers. In this study, the convenient and accurate carrier tests for the evaluating virucidal activities toward viruses were established. Moreover, this study highlighted that longer exposure time or higher concentration of disinfectants compared to the wiping method was required to inactivate the virus on surfaces with the dropping method.

In Chapter 2, slightly acidic hypochlorous acid waters (SAHWs) containing different concentrations of free available chlorine (FAC) were evaluated for their virucidal activity toward IBV and a low pathogenic H7N1 avian influenza virus (AIV) in liquid, on surfaces and in the air, with the presence of organic materials. In suspension test, abiotic carrier test, and direct spray test, SAHWs containing different concentrations of FAC - 62, 119, 220, 300, and 540 ppm (SAHW-62, -119, -220, -300, and -540, respectively) were utilized. In aerosol disinfection tests, IBV containing 0.5 % FBS was sprayed and exposed to SAHWs containing of FAC - 100, 200, 300 and 500 ppm (SAHW-100, -200, -300 and -500, respectively) for a few seconds in a closed chamber, before reaching the air sampler. In the suspension test, SAHW-62 could decrease the viral titer of both IBV and AIV by more than 1000 times (an effective level) within 30 sec. In the carrier test with the dropping technique, IBV on carriers showed high resistance to SAHWs, while AIV on plastic carrier was inactivated to an effective level within 1 min. With the wiping technique, SAHW-62 could inactivate both IBV and AIV on wiped plastic carriers to an effective level within 30 sec. However, SAHW-220 could not inactivate IBV in the wiping rayon sheet to an effective level. In the direct spray test, sprayed SAHW-540 within 20 min, and SAHW-300 within 10 min, could inactivate IBV and AIV on the rayon sheets to undetectable level, respectively. In the aerosol disinfection tests, IBV exposed to SAHW-100 and -200 for a few seconds decreased by 0.21 log_{10} and 0.80 log_{10}, respectively, compared to the pre-exposed samples to SAHWs as controls. On the other hand, reductions of 1.16 log_{10} and 1.67 log_{10} were achieved following the exposure to SAHW-300 and -500, respectively, within a few seconds. This study indicates that the usage of wipes with SAHWs could eliminate viruses from contaminated carriers, while viruses remained in the wipes. Besides, a small volume of sprayed SAHW is potentially effective toward the viruses on the rayon sheets for daily cleaning in the application area. In addition, SAHWs have rapid in vitro virucidal activity toward aerosolized IBV.

In Chapter 3, the epidemiology of viral pathogens indicators of biosecurity levels in pig farms was investigated. Porcine sapelovirus (PSV) and mammalian orthoreoviruses (MRVs) were used as the indicators on two pig farms. Although 138 PSVs were detected from a total of 199 fecal specimens of healthy pigs collected from two farms in Japan, no PSV was isolated. On the other hand, 12 MRVs were detected and 10 were isolated from the collected feces in one of two farms. The detection of MRVs has declined after June 2020, probably due to adoption of disinfection with QAC diluted with 0.17% $FdCa(OH)_2$ solution (Mix-500) that prevented MRVs infection among pigs. By sequencing based on the partial S1 gene of MRVs, MRV isolates were classified as MRV1 and MRV2. Additionally, the virucidal activities of disinfectants toward the isolated MRV1 were evaluated by the suspension and carrier tests in the presence of organic materials likewise the method used in Chapter 1. The evaluated disinfectants were the following: QAC-500,

FdCa(OH)$_2$, Mix-500, sodium hypochlorite at 100 ppm of total chlorine (NaClO-100) and NaClO-1000. In the suspension test, all disinfectants except for NaClO-100 efficiently inactivated MRV1 within 1 min. In the carrier test with the dropping technique, 0.17% FdCa(OH)$_2$, Mix-500 and NaClO-1000 required 5 min to efficiently inactivate MRV1, whereas it took 30 min for QAC-500 to efficiently inactivate the virus. With the wiping technique, Mix-500 and NaClO-1000 could inactivate MRV1 below the detection limit on the carrier and to the effective level in the wiping-sheet, respectively, within 30sec. The results of this study suggest that PSV are widespread in pig farms in Japan. On the other hand, although different serotypes of MRVs are circulating among pigs, the occurrence of MRVs in the farms decreased as a result of the disinfection using Mix-500.

In this study, convenient and accurate evaluation methods of virucidal activity of disinfectants have been established and utilized. Moreover, the virucidal activity of SAHWs toward viruses has been evidenced by means of tests simulating the practical application of appropriate disinfectants. In addition, although PSV infection among pigs could not be controlled, effective disinfection prevented MRVs infection, which suggests that further development of effective strategy is required to reduce PSV. It is considered that this research contributes to potential benefits to the livestock industry from a biosecurity perspective and reduces the occurrence of pathogens.

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