Development of a Risk Factor Surveillance System for Conservation of Endangered Bird Species and Studies on the Risk for their Population Reduction Due to Avian Influenza Virus Infection and a Countermeasure against the Risk

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## [Abstract]

Key Words: Highly pathogenic avian influenza, Surveillance system, Anti-influenza virus drug, Cultured cell, Endangered bird species, Evaluation of susceptibility, Risk evaluation, Infectious diseases, Lead poisoning

Endangered birds are threatened by avian influenza epidemics and low-level lead exposure. Therefore, the purpose of this project was to develop a comprehensive risk assessment method for infectious diseases and environmental pollution and to promote conservation measures for endangered birds. The development of such a method would also help to facilitate the implementation of avian influenza monitoring in East Asia, including Japan, the promotion of prompt countermeasures, and the strengthening of lead bullet regulations outside of Hokkaido based on risk assessments of lead contamination.

The project comprises three themes. In the theme 1, we summarized the overall research, focusing on highly pathogenic avian influenza. Then, we developed a comprehensive risk assessment methodology considering low-level lead exposure, which causes immunosuppression. In addition, we proposed a method for social implementation of this methodology. We also proposed a socially implementable risk assessment method as an outcome by (i) using a highly effective method developed with reference to practical examples in Japan and overseas and (ii) conducting simulations at a model site. Furthermore, we conducted active surveillance at waterfowl wintering grounds, performed risk assessment workshops for government veterinarians, and proposed a comprehensive risk assessment method that can be socially implemented in Japan.

In the theme 2, we developed an effective new rapid diagnostic method, technology for early detection of the virus, and environmental cleanup technology, with the aim of establishing countermeasures against highly pathogenic avian influenza virus (HPAIV) infection in endangered birds. In addition, we elucidated the HPAIV susceptibility of endangered species and common species that share habitats with them and developed a method for evaluating susceptibility in endangered bird species by using cultured cells, thereby establishing a basis for risk assessment and infection prevention in endangered bird species. In addition, we obtained knowledge that will provide the basis for developing treatment methods for endangered birds infected with HPAIV. We also established the utility of environmental water in the early detection of HPAIV as well as efficient methods for its detection by demonstrating that ducklings, pintails, and mallards infected with recent domestic isolates shed the virus for a period of time asymptomatically and serve as a source of infection for endangered bird species. We infected cultured cells of 11 bird species with HPAIV and observed the expression pattern of the antiviral Mx gene and found that the expression pattern of the chicken, a model bird species with high mortality, was similar to that of four endangered raptor species (bear hawk, golden eagle, goshawk, falcon) as well as the Okinawa rail. This method enabled the non-invasive estimation of HPAIV susceptibility in endangered bird species.

In the theme 3, we aimed to determine the status of lead contamination in raptors outside of

Hokkaido via lead bullets and other sources and to clarify the direct (i.e., the development of lead poisoning) and indirect (i.e., changes in susceptibility to HPAIV) risks to raptors from the use of lead bullets. We identified areas where lead poisoning and low-level lead exposure are likely to affect populations and made policy recommendations regarding areas where restrictions on the use of lead bullets and other materials should be prioritized as an outcome. In addition, we were the first in the world to demonstrate immunosuppression caused by lead exposure at the gene expression level. Based on these results, we were not only able to reveal specific areas where raptors are at high risk of lead exposure, but also to show that raptors may be at higher risk of lead exposure because of the higher number of hunters compared with other areas around the site where HPAIV was isolated from dead raptors.

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