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Molecular Sex Identification Based on Length Dimorphisms between *ZFX* and *ZFY* Genes in Livestock Species

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Precise, rapid, and simple methods for sex typing in animals are one of most important techniques in livestock industry and research fields including forensic science. In this study, based on the presence or absence of interspersed repeat (IR) sequence between X and Y chromosomes, two sets of amplification primers were tested for molecular sex identification in six mammalian livestock species (pig, red deer, goat, sheep, cattle, and horse). Sexual dimorphisms between male and female animal DNA samples were easily detected from PCR analyses. The amplification of the *ZFX* and *ZFY* genes produced two distinct patterns on the agarose gels in all species tested, reflecting sex chromosome dimorphisms based on a length difference between XX and XY. The amplification products of males showed two distinct heteroduplex bands amplified from *ZFX* and *ZFY*, and no *ZFY* band was found in all female DNA samples. *ZFY* amplicons were longer in length than those of *ZFX* except for intron 9 amplicons of pig and horse. The sex types by *ZFX-ZFY* PCR analysis provided the identical information to those of amplification of the Y chromosome-specific SRY gene and those of investigations on phenotypic genders in each species. Application of this PCR analysis can be useful and provided rapid, simple, and clear information on the sexes for various tissue samples originated from livestock species tested.

Key words: *ZFX, ZFY*, sexual dimorphism, interspersed repeat, livestock species

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Development of Cold-adapted Live Vaccine against H9N2 Influenza Virus

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H9N2 influenza viruses have been circulating in chickens in Korea since 1996. These viruses are endemic in poultry during the past decade and cause considerable economic losses by reducing egg production and about 10-30% mortality. These clinical signs prompted us to develop a cold-adapted vaccineto protect chickens from infections of H9N2 influenza viruses. To develop the effective live attenuated vaccine against H9N2 viruses, we adapted wild-type A/Chicken/Korea/S21/03 (H9N2) in chicken embryo fibroblast cells to grow at 25℃ by gradually lowering temperatures from 33℃ to 25℃. When we compared genetic composition of cold-adapted H9N2 virus with that of wild-type virus, total 24 amino acids were mutated in cold-adapted viruses. In order to confirm that cold-adapted live H9N2 vaccine is safe in chickens, we intranasally inoculated 10 layers with 10^6EID_{50}(1ml) of cold-adapted or wild-type H9N2 viruses. Layers inoculated with cold-adapted H9N2 viruses did not show any clinical signs such as edema of face, mild to severe respiratory signs, reduced egg production, and 20% mortality compared to layers infected with wild-type H9N2 viruses. The protective efficacy of cold-adapted H9N2 live vaccine was confirmed by challenging vaccinated 10 layers with wild-type H9N2 viruses. The vaccinated layers with cold-adapted H9N2 viruses did not show clear clinical signs compared to unvaccinated control layers. Our study indicates that cold-adapted live H9N2 vaccine may be used for controlling H9N2 influenza viruses in chickens.

Key words: Cold-adapted live vaccine, H9N2 influenza virus