別紙様式第４

学　位　論　文　要　旨

博士課程 ㊙・乙	第21号	氏　名	NGUYEN THI TRANG

[論文題名] **Detection of porcine reproductive and respiratory syndrome virus in naturally infected pigs.**

1. Detection of porcine reproductive and respiratory syndrome virus in oral fluid from naturally infected pigs in a breeding herd.

Nguyen Thi Trang, Takuya Hirai, Tsukasa Yamamoto, Mari Matsuda, Naoko Okumura, Nguyen Thi Huong Giang, Nguyen Thi Lan, Ryoji Yamaguchi.

(Journal of Veterinary Science, 15(3):361-367, 2014)

2. Enhanced detection of Porcine reproductive and respiratory syndrome virus in fixed tissues by in situ hybridization following tyramide signal amplification

Nguyen Thi Trang, Takuya Hirai, Pham Hong Ngan, Nguyen Thi Lan, Naoyuki Fuke, Keiko Toyama, Tsukasa Yamamoto, Ryoji Yamaguchi.

(Journal of Veterinary Diagnostic Investigation, 27 (3), May 2015, in press)

[要　旨] The objectives of the present study were first to evaluate the anatomic localization of porcine reproductive and respiratory syndrome virus (PRRSV) in naturally infected pigs and to determine whether oral fluid could be used to detect the virus in infected animals. Secondly, to evaluate the sensitivity of biotinyl-tyramide-based in situ hybridization (TISH) method by comparison with chromogenic in situ hybridization (CISH) and immunohistochemistry (IHC) methods. Thirdly, to determine the effect of fixative and fixation time on the detection of porcine reproductive and respiratory syndrome virus (PRRSV) in paraffin-embedded tissues. Lung samples were fixed in 4% paraformaldehyde (PFA) or 10% neutral buffered formalin (NBF) for various times before paraffin embedding. PRRSV in sera and oral fluid were identified by nested reverse transcription PCR (nRT-PCR) while lung, tonsil, and tissue associated with oral cavity were subjected to nRT-PCR, immunohistochemistry (IHC), and *in situ* hybridization (ISH). These results confirm previous findings that PRRSV primarily replicates in tonsils and is then shed into oral fluid. Therefore, oral fluid sampling may be effective for the surveillance of PRRSV in breeding herds. TISH can detect PRRSV RNA in paraffin-embedded tissues after up to 90 days of fixation. PRRSV nucleic acids and antigens were better preserved in 4% PFA than in 10% NBF. Compared with CISH and IHC testing methods, TISH appeared to be more sensitive for the detection of PRRSV in paraffin-embedded tissues.

Keywords: In situ hybridization, lung, oral fluid, porcine reproductive and respiratory syndrome virus, tonsil.