Unexpected Strangles in Imported Horses: Molecular Identification of *Streptococcus equi* subsp. *equi*

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Abstract

Background: Strangles is a highly contagious equine respiratory disease caused by *Streptococcus equi* subsp. *equi* (SEE) and is characterized by fever, mucopurulent nasal discharge, and abscess formation in the submandibular and retropharyngeal lymph nodes. In contrast, *Streptococcus equi* subsp. *zooepidemicus* (SEZ) is a commensal and opportunistic pathogen. Young horses aged 1–5 years are most susceptible, particularly under stress such as transportation or overcrowding. Strangles is currently included among the diseases that must be certified negative prior to importation into Thailand. However, the disease has previously been reported in association with imported horses. In 2014, a SEE outbreak was documented in horses imported from Europe.

In September 2025, several horses were imported into Thailand for a competition scheduled for December. During quarantine, a yearling horse showed clinical respiratory signs with nasal discharge and a submandibular abscess. After a week in the same location, ten additional horses developed similar symptoms. Then the horse's owner notified DLD to investigate the situation.

Objectives: To investigate a suspected outbreak in a horse herd and confirm SEE as the causative agent of strangles in imported horses using bacteriological and molecular diagnostic methods.

Methods: Thirty-five sera were collected from the horse herd showing clinical respiratory signs. Then, the sera were subjected to serological testing by ELISA. Further sampling included 52 whole bloods, 5 pus swabs, 3 wound swabs, and 23 nasal swabs from seropositive herds, which were subjected to bacterial isolation and direct real-time PCR targeting the *SIR2* gene. Subsequently, suspected colonies were confirmed by conventional PCR targeting both the *SeM* and *SIR2* genes. Then, Sanger sequencing of universal *16S rRNA*, *SeM,* and *SIR2* genes was performed for confirmation. Furthermore, antimicrobial susceptibility of confirmed isolates was tested by disc diffusion. Epidemiological investigation included two additional farms with a history of horse import from the same batch.

Results: Seropositivity to SEE was 48.6% (17/35 samples). Bacterial isolation was obtained primarily from pus swabs, whereas most nasal swabs were culture-negative despite positive molecular detection. Isolates produced small, beta-hemolytic colonies on blood agar, and microscopic examination revealed Gram-positive cocci consistent with *Streptococcus* spp.

Direct real-time PCR targeting the *SIR2* gene detected SEE in clinical samples, while conventional PCR targeting both *SeM* and *SIR2* genes confirmed the identity of cultured isolates, with results confirmed by Sanger sequencing (100% sequence identity). These findings indicate that pus swabs with 80% positive (4/5 samples) are more suitable for bacterial isolation, whereas nasal swabs with 4.3% positive (1/23 samples) are reliable for molecular detection. For the horse with a positive nasal swab, the corresponding serum sample was also positive by ELISA. No additional samples were collected from this animal as the clinical condition did not improve, and the horse was subsequently euthanized. The 52 blood samples and 3 wound swabs were all negative. Additionally, SEEs were most susceptible to antibiotics, and horses recovered after treatment. Furthermore, one of two additional farms that had imported horses from the same source was investigated, and clinical samples from these farms were also positive for SEE.

Conclusion: This study confirms SEE infection in imported horses in Thailand and highlights the risk of post-import disease introduction. Pus swabs were more suitable for bacterial isolation, whereas nasal swabs were reliable for molecular detection. The use of *SIR2*-targeted real-time PCR enabled specific detection, while dual-target PCR improved diagnostic confidence. Molecular methods demonstrated higher sensitivity than culture, and epidemiological findings suggest that horse movement contributed to disease transmission between farms. These findings emphasize the importance of strict post-arrival quarantine protocols, active surveillance, and integrated diagnostic approaches for effective control of transboundary equine diseases.

Keywords: Equine, PCR, Strangles, *Streptococcus equi* subsp. *equi*

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