ISWAVLD 2023
International Symposium of the World Association of Veterinary Laboratory Diagnosticians
29 JUNE-1 JULY 2023
Congress Centre Lyon
Towards the veterinary diagnostics of the future

Main topic : Surveillance and control of emerging diseases

Serodiagnostic tests using OmpS1 and OmpS2 proteins as antigens for the detection of Salmonella enterica subsp. enterica serovar Abortusequi in carrier horses

KINOSHITA Y. [1], *NIWA H.* [1], *UCHIDA-FUJII E.* [1], *UENO T.* [1]

[1] *Equine Research Institute, Japan Racing Association, Shimotsuke, Japan*

Introduction
Salmonella enterica subsp. *enterica* serovar Abortusequi is a host-adapted bacterium in equines that causes abortion in mares and neonatal septicemia. In recent years, outbreaks of *S.* Abortusequi infection have occurred worldwide, including in Italy, Argentina, China and Japan. Although it is important to identify carrier horses to prevent the spread of *S.* Abortusequi, it is often difficult to determine whether a horse is a carrier or not using serologic testing with the conventional agglutination test. In this study, we developed peptide enzyme-linked immunosorbent assays (ELISAs) targeting the Salmonella porins OmpS1 and OmpS2, and evaluated the applicability of the ELISAs.

Methods
The amino acid sequence diversity of OmpS1 and OmpS2 was investigated in 21 strains of *S.* Abortusequi, including 16 domestic Japanese strains and 5 non-Japanese strains. We synthesized six and seven peptides of 30 amino acid residues for OmpS1 and OmpS2, respectively, and constructed indirect ELISAs (13 in total) using each synthesized peptide as an antigen. Each indirect ELISA was validated by using sera from both clinically healthy Thoroughbred horses and mares aborted due to *S.* Abortusequi infection. The OD values of each indirect ELISA were then determined using sera from horses with high agglutination titers including four horses confirmed to be carriers of *S*. Abortusequi by bacterial isolation from post-mortem samples and 27 sera from eight non-carrier horses.

Results
All 21 *S*. Abortusequi strains possessed the OmpS1 and OmpS2 genes, and the amino acid sequences showed high identities: OmpS1 was completely identical among the 21 strains, while OmpS2 differed by only six residues out of 400 amino acids between Japanese and non-Japanese strains. Although each indirect ELISA with healthy horse sera showed hardly any increase in OD values, the OD values in aborted horses increased in most cases either before or after abortion. Agglutination titers for the four carrier horses confirmed at euthanasia were 1:1,280, and for the non-carrier horses, seven were 1:1,280 and one was 1:640. The median OD values from the 13 indirect ELISAs ranged from 0.34-0.61 for the carrier horses and 0.08-0.13 for the non-carrier horses at euthanasia. In addition, for the samples collected from the non-carrier horses over time, the OD values of the indirect ELISAs tended to decrease or remain low, even when the aggregation titer was greater than 1:1,280.

Conclusions
OmpS1 and OmpS2 are highly conserved by *S*. Abortusequi, and antibody detection based on OmpS1 and OmpS2 antigens could be used to distinguish *S* . Abortusequi-infected horses from healthy horses and also carriers from non-carriers.