KJVS
Korean Journal of Veterinary Service
https://doi.org/10.7853/kjvs.2023.46.4.263
pISSN: 1225-6552 eISSN: 2287-7630
Korean J Vet Serv 2023;46(4):263-268

ORIGINAL ARTICLE

Evaluation of concurrent immunizations with equine influenza virus and strangles vaccines

Dong-Ha Lee[1,2], Kyungmin Jang[1], Taemook Park[1], Youngjong Kim[1], Kyoung Hwan Kim[1], Eun-bee Lee[2], Young Beom Kwak[3], Eun-Ju Ko[2]*

[1]Racing Resources Management Team, Jeju, Korea Racing Authority, Jeju 63066, Korea
[2]Department of Veterinary Medicine, College of Veterinary Medicine, Jeju National University, Jeju 63243, Korea
[3]Racing laboratory, Jeju, Korea Racing Authority, Jeju 63066, Korea

Despite regular vaccinations, equine influenza virus (EIV) and *Streptococcus equi* subsp. *equi* (strangles) are the cause of highly contagious respiratory infections in horses. Many recent studies have reported that the concurrent administration of two vaccines could simplify horse management and minimize veterinary expenses. However, there is little information available regarding the efficacy of concurrent vaccinations against EIV and strangles. In this study, we evaluated EIV-specific antibody responses following the single EIV vaccination with the recombinant viral-vectored EIV vaccine or concurrent vaccination with the EIV and inactivated strangles vaccines. Blood samples were collected at 1-, 2-, 4-, and 8 weeks post-immunization (wpi) from each group. EIV-specific antibodies were evaluated by enzyme-linked immunosorbent assay (ELISA) and hemagglutination inhibition (HAI) assay. Both single and concurrent vaccination showed similar levels of EIV-specific serum immunoglobulin g (IgG) at 1 and 2 wpi. However, at 4 to 8 wpi, the EIV-only vaccination group showed significantly higher serum IgG levels than those from the concurrently vaccinated group. The HAI titers showed similar trends as the ELISA data, except at 8 wpi when both groups presented HAI titers with no significant differences. These data demonstrate that the concurrent vaccination against EIV and strangles could compromise the humoral immune response to equine influenza between vaccination intervals, which suggests the use of the consecutive vaccination protocol for EIV and strangles rather than concurrent vaccination.

Key Words: Equine influenza virus, Strangles, Concurrent vaccination, Immunoglobulin G, Hemagglutination inhibition titer

Received November 12, 2023
Revised December 7, 2023
Accepted December 7, 2023

Corresponding author:
Eun-Ju Ko
E-mail: eunju@jejunu.ac.kr
https://orcid.org/0000-0002-1081-904X

INTRODUCTION

Equine influenza virus (EIV) and strangles are the most common causes of contagious respiratory infections in horses. EIV belongs to the family Orthomyxoviridae, and the genus Influenza A (Lee et al, 2022). EIV infection can cause anorexia, cough, nasal discharge, pyrexia, secondary bacterial infections (Pavulraj et al, 2021; Lee et al, 2022). The EIV infection is mainly caused by two subtypes of influenza A viruses, namely H7N7 and H3N8. The H7N7 subtype has not been isolated since the 1970s. In unvaccinated horses, clinical signs can be observed 48 h after EIV infections (Paillot 2014). Strangles is a contagious upper respiratory tract infection of equidae caused by *Streptococcus equi* subsp. *equi* (*S. equi*) (Lee et al, 2020). It affects horses of all ages but is more common in weanlings or yearlings (Lee et al, 2020). *S. equi* can spread quickly, especially in naïve populations, which necessitates the implementation of movement restrictions and the cancellation of equestrian events (Allkofer et al, 2021). Additionally, because of the high morbidity caused by strangles infections, strict prevention protocols including disease surveillance, quarantine of horses, and regular vaccination programs

need to be implemented, which result in significant financial losses to the equine industry (Paillot 2014).

Various EIV and strangles vaccines are available globally, including inactivated (killed), subunit, and recombinant virus-vectored vaccines. In South Korea, the recombinant canarypox virus-vectored EIV (ProteqFlu®, Boehringer Ingelheim, Ingelheim am Rhein, Germany) and inactivated strangles vaccines (Equivac® S, Zoetis Inc. Parsippany, NJ, USA) are currently used. The ProteqFlu® vaccine contains two recombinant canarypox viruses expressing the hemagglutinin (HA) gene from the equine influenza virus strains A/eq/Ohio/03 (H3N8) and A/eq/Richmond/1/07 (H3N8) (Lee et al, 2022). According to the manufacturer's protocol, 6-month-old horses should receive two initial primary vaccinations at intervals of 4~6 weeks, followed by a second dose 5~6 months later. Booster shots are to be administered to the horses every 6 months (Cho et al, 2020). The Equivac® S vaccine is administered intramuscularly to horses for protection against strangles. The foal receives the first vaccination at 3 months, followed by a second and third vaccination at 2-week intervals. Booster vaccinations are given annually. Presently, the vaccines are administered concurrently. However, there is no information regarding the immune responses induced by concurrent vaccination with the ProteqFlu® against EIV and Equivac® S vaccines against strangles in horses.

Concurrent vaccination of both vaccines has been applied to decrease veterinary expenses and reduce repeated handling associated stress to the horses (Allkofer et al, 2021). Nevertheless, according to previous studies, the efficacy of concurrent administration remains controversial because of interference due to antigenic competition and various other factors (Gildea et al, 2016, Ohta et al, 2020).

Therefore, in this study, we aimed to compare the EIV-specific immune responses induced by the concurrent administration of a recombinant canarypox EIV vaccine (ProteqFlu®) and an inactivated strangles vaccine (Equivac® S) with those induced by a single EIV vaccine in horses.

MATERIALS AND METHODS

Animals

Twelve Jeju ponies (2~10 years of age) were used in this study and were randomly divided into two groups. The horses were vaccinated against the EIV every six months and against strangles annually. All horse experiments were performed according to the guidelines of the Korea Racing Authority (KRA) and approved by the Institutional Animal Care and Use Committee (IACUC) protocol (protocol number KRA IACUC-2316).

Reagents

Bovine serum albumin (Thermo Fisher Scientific, Waltham, Ma, USA) and Tween20 (Sigma-Aldrich, St. Louis, MO, USA) were used in the enzyme-linked immunosorbent assay (ELISA). The receptor-destroying enzyme (RDE) was purchased from Denka Seiken (Chuo, Tokyo, Japan) and used in the hemagglutination inhibition (HAI) assay. All reagents were prepared according to the manufacturer's instructions.

Vaccines

The recombinant canarypox EIV vaccine ProteqFlu® and inactivated strangles vaccine Equivac® S were used in this study. The EIV vaccine contained two recombinant canarypox viruses expressing the hemagglutinin (HA) genes of equine influenza virus strains A/eq/Ohio/03 (H3N8) (American strain, Florida sublineage clade 1) and A/eq/Richmond/1/07 (H3N8) (American strain, Florida sublineage clade 2) adjuvanted with carbomer. The strangles vaccine contained *S. equi* inactivated cell-free acid extract adjuvanted with aluminum hydroxide. The total protein concentration of each vaccine was measured using a DS-11 spectrophotometer (DenoVix Inc., Wilmington, DE, USA) and they were stored at −80℃ until analysis.

https://doi.org/10.7853/kjvs.2023.46.4.263

Immunization

Six horses were immunized intramuscularly with only the EIV vaccine, whereas six other horses were concurrently immunized with EIV and the strangles vaccines through the same administration route. Blood samples were collected 1-, 2-, 4-, and 8- weeks post-immunization (wpi) from each group (Fig. 1). During immunization, no horses presented adverse effects, including swelling at the injection site. Sera were isolated from the blood samples by centrifugation and stored at −20℃ until analysis.

Enzyme-linked immunosorbent assay (ELISA)

To measure the EIV-specific IgG levels in the sera, serially diluted sera were added to A/eq/Miami/2/63 (H3N8)-coated ELISA plates (400 ng/well) after blocking. Horseradish peroxidase labeled anti-horse IgG was used to detect EIV-specific IgG in the equine sera. After the addition of 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution, the reaction was then stopped by a 0.16 M sulfuric acid stop solution. Optical density (OD) was measured at 450 nm using a plate reader (Molecular Devices, Sunnyvale, CA, USA).

Hemagglutination inhibition (HAI) assay

The HAI assay was performed by a previously described method (Lee et al, 2022). Briefly, 10 μL of sera and 30 μL of RDE were mixed and incubated at 37℃ for 18 h and then inactivated by heat treatment at 56℃ for 30 min. The sera were serially diluted two-fold (final volume of 25 mL) and incubated with 8 hemagglutination units of A/Equine/2/Miami/63 H3N8 virus (final volume of 25 mL) in U-bottom plates for 30 min. Chicken red blood cells (50 mL, 0.5%) were added to the plates and the HAI titers were determined after 40 min.

Statistical analysis

All results are presented as the mean±standard error of the mean (SEM). Statistical significance was determined using the Student's t-test. Statistical significance was set at $P<0.05$. All data were analyzed using Prism software 10.0.2. (GraphPad Software, San Diego, CA, USA).

RESULTS

EIV-specific serum IgG levels

We immunized the horses with the EIV vaccine alone or in combination with the strangles vaccine to evaluate the effects of the concurrent EIV and strangles vaccination on the EIV-specific serum IgG levels. After immunization, sera were collected on days 1-, 2-, 4-, and at 8-weeks, and the EIV-specific serum IgG antibodies were quantified using ELISA (Fig. 2).

Both single and concurrent immunization showed similar levels of EIV-specific serum IgG at 1- and 2-weeks post-immunization (wpi), respectively (Fig. 2A, 2B). However, at 4 to 8 wpi, the EIV vaccine-only group showed higher serum IgG levels than the concurrently immunized group, (Fig. 2C, 2D) with a significant difference between the groups. ($P<0.001$ and $P<0.01$, respectively). These results suggest that concurrent EIV and strangles vaccination could compromise the EIV-specific serum IgG levels.

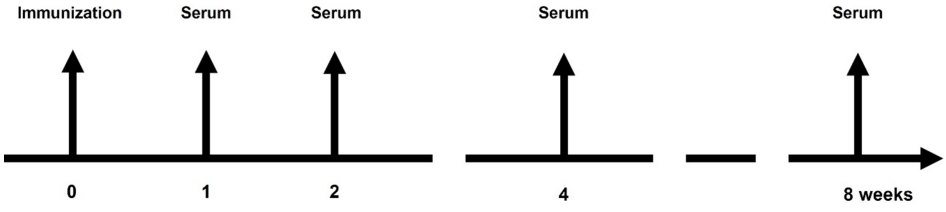

Fig. 1. Timeline of immunization and sample collection from horses. Arrows denote immunization and blood sampling time points. Horses were immunized with equine influenza virus (EIV) alone or concurrently with EIV and strangles vaccines.

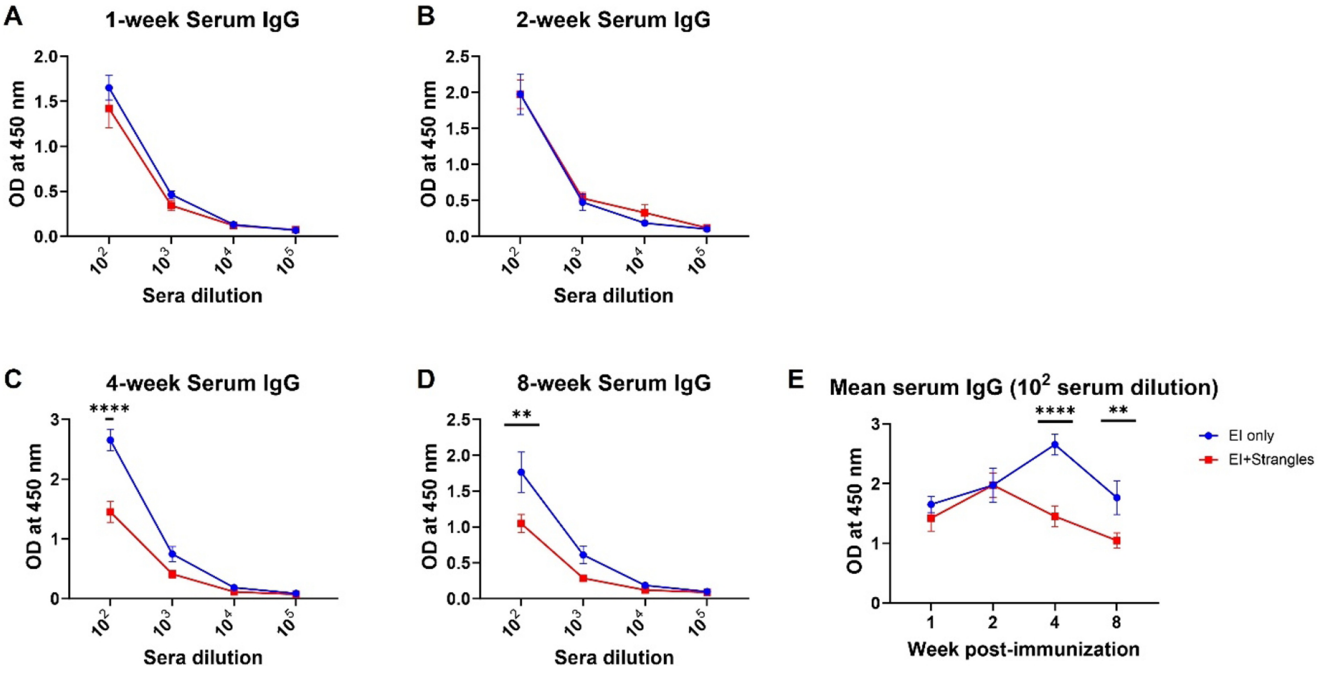

Fig. 2. Equine influenza virus (EIV)-specific IgG antibody levels in serum after immunization. Horses were immunized with EIV vaccine alone or concurrently with EIV and strangles vaccine. The serum was taken 1, 2, 4, and 8 weeks post immunization and the EIV-specific IgG antibody levels were measured by enzyme-linked immunoabsorbent assay (A～D). The mean serum IgG level was 10^2 times serum dilution (E) $^{**}P<0.01$; and $^{****}P<0.001$ as indicated between the groups.

HAI levels against EIV

Both single and concurrent immunization elicited HAI titers with no significant differences at 1 and 2 wpi, respectively (Fig. 3A, 3B). At 4 wpi, the HAI titers in the EIV vaccine-only group were sustained and showed significantly higher levels than that of the concurrently immunized group ($P<0.05$) (Fig. 3C). The HAI titers showed similar trends as the ELISA data, except for the 8 wpi results in which both groups elicited similar HAI titers (Fig. 3D). These results suggest that the concurrent immunization may not have a beneficial effect in increasing HAI titers.

DISCUSSION

In this study, we evaluated the EIV-specific antibody responses between a group of horses that was concurrently immunized with EIV and strangles vaccines and a group that was immunized with the EIV vaccine alone. Both single and concurrent vaccination showed similar levels of EIV-specific serum IgG and HAI titers at 1 and 2 wpi, respectively. However, at 4 wpi, the EIV-only vaccination group showed higher serum IgG and HAI titer levels than the concurrently vaccinated group, with a significant difference between the groups ($P<0.001$ and $P<0.05$, respectively). This result contradicts the results of Lee et al., 2022 and Gildea et al., 2016, where horses immunized with concurrent vaccination on the same day had significantly higher antibody levels than those immunized with only the EIV vaccine. These differences could be attributable to the types of vaccines, adjuvants, and variations in the horse species between the experiments (Lee et al, 2022). Nevertheless, the results obtained from our study indicated that the concurrent immunization of recombinant canarypox vectored EIV and inactivated strangles vaccines negatively impacted the humoral response against EIV. Therefore, concurrent immunization with both vaccines is not recommended in Jeju Ponies. However, further investigations involving different species and larger populations are required to verify our results.

https://doi.org/10.7853/kjvs.2023.46.4.263

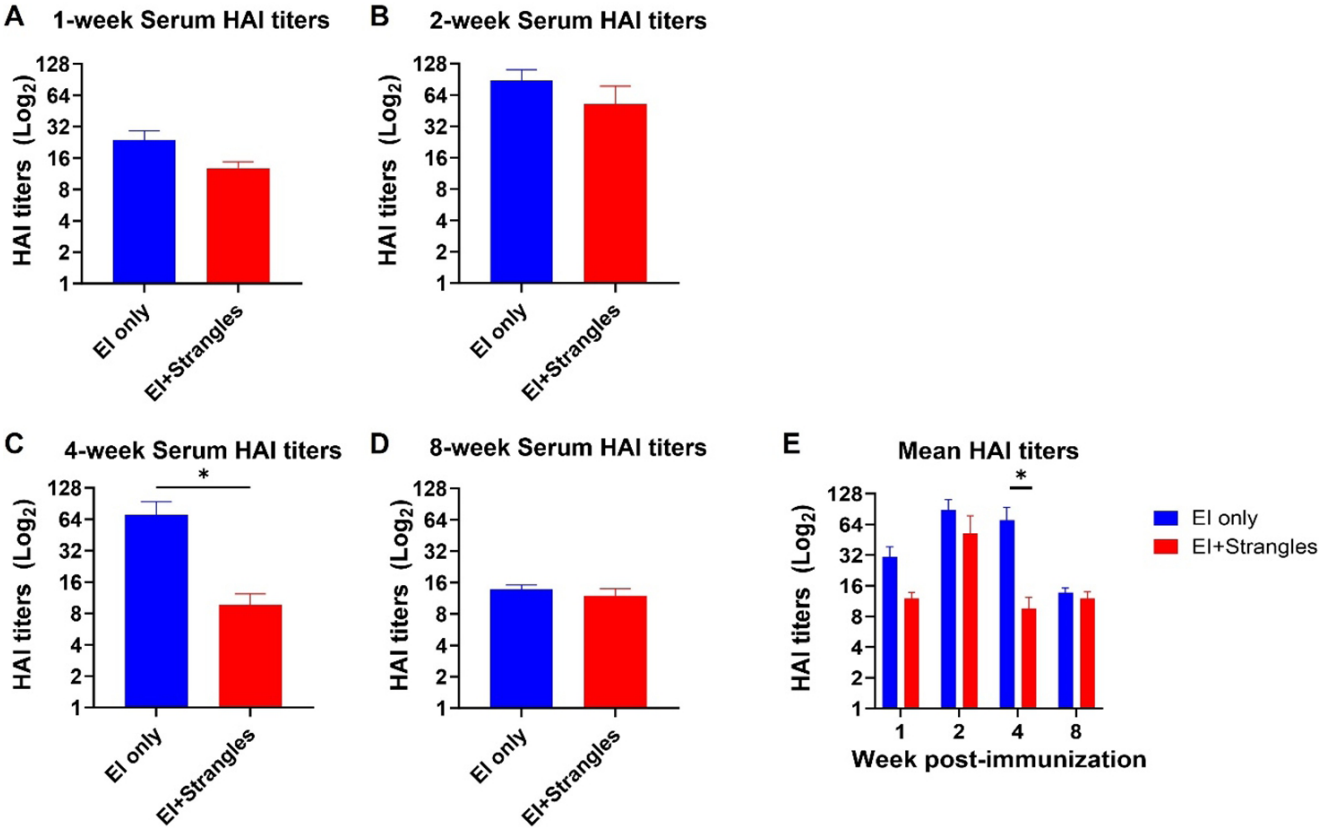

Fig. 3. Serum hemagglutination inhibition titers (HAI) against equine influenza virus (EIV). HAI titers were determined from the immune serum of EIV vaccine alone or concurrently with EIV and strangles vaccine. The serum was taken 1, 2, 4, and 8 weeks post immunization (A∼D). The mean HAI titers level was represented (E). All results were shown as mean±SEM. *P<0.05 as indicated between the groups.

In a comparative vaccine study carried out by Gildea et al., 2013, the administration of the EIV vaccine combined with a tetanus vaccine adjuvanted with carbomer and aluminum hydroxide elicited a superior antibody response in young Thoroughbred horses. The study suggested that the administration of two adjuvants may be of benefit in increasing the antibody response, but our results contradicted this because the EIV and the strangles vaccines used in our study also included carbomer and aluminum hydroxide, respectively. Therefore, we propose that the inclusion of two adjuvants does not always induce enhanced antibody responses, and the decreased antibody response induced in our study may be related to the interference caused by antigenic competition between EIV and *S. equi*. However, further investigation is needed to elicit more comprehensive data.

Most of the EIV vaccine efficacy studies conducted so far have evaluated humoral responses using a single radial hemolysis assay or HAI titers. This is because the evaluation of cell-mediated immune (CMI) responses in horses is complex and difficult to measure (Pavulraj et al, 2021; El-Hage et al, 2022). However, CMI plays an important role in clearing the EIV from the respiratory tract and protecting horses from EIV infection despite low levels of antibodies (Bryant et al, 2010). Therefore, an additional evaluation of whether concurrent immunization would negatively impact cellular responses should be included before continuing the immunization. This could be done by measuring EIV-specific interferon-gamma after a viral challenge.

In conclusion, even though the concurrent immunization with recombinant canarypox vectored EIV vaccine and inactivated strangles vaccine can minimize veterinary intervention and the cost associated with immuni-

zation, our data demonstrated that such concurrent immunization reduced the humoral responses against EIV. Therefore, consecutive immunization or an improved immunization protocol may need to be considered for reducing the burden of EIV infection outbreaks in horses.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ORCID

Dong-Ha Lee, https://orcid.org/0000-0002-8898-3632
Kyungmin Jang, https://orcid.org/0009-0005-6380-7308
Taemook Park, https://orcid.org/0000-0003-4408-6048
Youngjong Kim, https://orcid.org/0000-0001-9421-5333
Kyoung Hwan Kim, https://orcid.org/0000-0003-4259-7872
Eun-bee Lee, https://orcid.org/0000-0003-3654-5654
Young Beom Kwak, https://orcid.org/0000-0003-0769-1572
Eun-Ju Ko, https://orcid.org/0000-0002-1081-904X

REFERENCES

Allkofer A, Garvey M, Ryan E, Lyons R, Ryan M, Lukaseviciute G, Walsh C, Venner M, Cullinane A. 2021. Primary vaccination in foals: a comparison of the serological response to equine influenza and equine herpesvirus vaccines administered concurrently or 2 weeks apart. Arch Virol 166: 571-579.

Bryant NA, Paillot R, Rash AS, Medcalf E, Montesso F, Ross J, Watson J, Jeggo M, Lewis NS, Newton JR. 2010. Comparison of two modern vaccines and previous influenza infection against challenge with an equine influenza virus from the Australian 2007 outbreak. Vet Res 41.

Cho MS, Lee JY, Lee SK, Song JY, Lee J, Hyun BH, Cho SD, Ouh IO. 2020. Antibody responses after vaccination against equine influenza virus in the Republic of Korea in 2019. J Prev Vet Med 44: 132-135.

El-Hage C, Hartley C, Savage C, Watson J, Gilkerson J, Paillot R. 2022. Assessment of humoral and long-term cell-mediated immune responses to recombinant canarypox-vectored equine influenza virus vaccination in horses using conventional and accelerated regimens respectively. Vaccines 10: 855.

Gildea S, Quinlivan M, Murphy BA, Cullinane A. 2013. Humoral response and antiviral cytokine expression following vaccination of thoroughbred weanlings—A blinded comparison of commercially available vaccines. Vaccine 31: 5216-5222.

Gildea S, Sanchez Higgins MJ, Johnson G, Walsh C, Cullinane A. 2016. Concurrent vaccination against equine influenza and equine herpesvirus-a practical approach. Influenza Other Respir Viruses 10: 433-437.

Lee DH, Birhanu BT, Lee EB, Lee SJ, Boby N, Park YS, Park SC. 2020. Pharmacokinetic and pharmacodynamic integration for optimal dosage of cefquinome against Streptococcus equi subsp. equi in foals. Vet Res 51:1-12.

Lee DH, Lee Eb, Seo Jp, Ko EJ. 2022. Evaluation of concurrent vaccinations with recombinant canarypox equine influenza virus and inactivated equine herpesvirus vaccines. J Anim Sci Biotechnol 64: 588.

Ohta M, Bannai H, Nemoto M, Kambayashi Y, Tamura N, Tsujimura K. 2020. Antibody responses against equine influenza virus induced by concurrent and by consecutive use of an inactivated equine influenza virus vaccine and a modified live equine herpesvirus type 1 vaccine in thoroughbred racehorses. J Equine Vet Sci 94: 103221.

Paillot R. 2014. A systematic review of recent advances in equine influenza vaccination. Vaccines 2: 797-831.

Pavulraj S, Bergmann T, Trombetta CM, Marchi S, Montomoli E, Alami SSE, Ragni-Alunni R, Osterrieder N, Azab W. 2021. Immunogenicity of Calvenza-03 EIV/EHV® vaccine in horses: comparative in vivo study. Vaccines 9: 166.

 https://doi.org/10.7853/kjvs.2023.46.4.263