Evaluation of antibody response induced by an oil-adjuvanted inactivated bivalent foot and mouth disease virus vaccine in cattle

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ABSTRACT

Foot-and-mouth disease (FMD), a globally significant viral disease of cloven-hoofed animals, can be managed by various strategies, including vaccination. The aim of this study was to assess antibody response in cattle that were vaccinated with a FMD vaccine. Two hundred and ten cattle from different herds (n=71) in the Black Sea Region of Türkiye were vaccinated with an oil-adjuvanted inactivated bivalent vaccine, which contained A_{22} Iraq and O_1 Manisa FMDV strains, and serum samples were collected from vaccinated cattle on day 28 following vac- cination. The antibodies produced in the vaccinated cattle were measured using solid-phase competition ELISA. Before vaccination, 53 samples had no detectable antibodies against serotype A, whereas 62 samples were found to be seronegative against serotype O (P=0.381). In the remaining samples, serotype A and serotype O antibody titres varied between 2.3 $\log_2$ and 3.6 $\log_2$, and 2.3 $\log_2$ and 3.3 $\log_2$, respectively. One hundred and sixty seven (79.5%) out of 210 vaccinated cattle had seroconverted to serotype A ($\log_2$ titre 3.8 to 5.6), whereas 159 (75.7%) cattle had neutralizing antibodies ($\log_2$ titre 3.5 to 5.6) against serotype O. There was a statistically significant difference in serotype A and serotype O antibody titres before and after vaccination (P=0.001). Additionally, after vaccination, the proportions of animals with protective titres ($\log_2$ titre $\geq$3.9) were significantly different between serotype A (152, 72.4%) and serotype O (130, 61.9%) (P=0.029). Compared to animals between 4-11 months, animals over 12 months had significantly higher mean neutralizing antibody titres for both serotypes O and A (P<0.001 for sero- types A and O). The results of this study revealed that the inactivated bivalent vaccine provided a reasonable level of protection against serotype A and serotype O. However, antibodies above the protection level against serotype O were relatively low in vaccinated cattle compared to serotype A. Post-vaccination monitoring should be utilized to evaluate the effectiveness of FMD vaccines.

Key words: foot and mouth disease; neutralizing antibody titres; post-vaccination monitoring; serotype A; serotype O; vaccine

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Introduction

Foot-and-mouth disease (FMD) is a viral disease that affects both domestic and wild cloven-hoofed animals, such as sheep, goats, swine, cattle, African buffalo, and deer (WOAH, 2022). Economic losses are caused by FMD because of the loss of meat and milk production, the death of young animals, and trade restrictions (KNIGHT-JONES and RUSHTON, 2013). Therefore, World Organisation for Animal Health (WOAH) classifies FMD as a notifiable disease (WOAH, 2022).

Vesicles on the mucus membranes of the mouth and nose, as well as on the hairless skin of the feet, are characteristic of the disease (GRUB- MAN and BAXT, 2004). Foot-and-mouth disease virus (FMDV, species Aphthovirus vesiculae) can also lead to asymptomatic, but persistent infection. Furthermore, animals that have been vaccinated or have naturally developed immunity are at risk of becoming persistently infected when exposed to infection (ALEXANDERSEN et al., 2002).

The etiological agent of the disease, FMDV, is a non-enveloped, single-stranded RNA virus with icosahedral capsid consisting of 60 copies of each of four different structural proteins, VP1 to VP4, and belongs to the genus *Aphthovirus* of the subfamily *Caphthovirinae* within the *Picornaviridae* family (ICTV, 2023). The G-H loop that is located between positions 140 and 160 of capsid protein VP1 has been deemed to be the primary site of antigenicity (VERDAGUER et al., 1995; DOMINGO et al., 2002). The lack of proofreading ability of FMDV's RNA-dependent RNA polymerase causes a high mutation rate during replication (DOMINGO et al., 2003). Antigenic diversity is a significant consequence of high mutation rates. Controlling FMD is very difficult due to the antigenic variation. FMDV has seven distinct serotypes, namely: Asia 1, A, C, O, SAT1, SAT2, and SAT3, with numerous subtypes (GRUBMAN and BAXT, 2004), and there is no cross-protection between serotypes and subtypes (MARTINEZ et al., 1988; AGGARWAL and BARNETT, 2002).

Although it has been eradicated in many regions of the world, FMD is still spreading in many countries in the Middle East, Africa, Asia, and South America (HAGAG et al., 2023; ASLAM and ALKHERAIJE, 2023; IRIARTE et al., 2023; ROMEY et al., 2023; WOLDEMARIYAM et al., 2023a).

FMD is an endemic disease in Türkiye, and biannual vaccination campaigns with inactivated vaccines are carried out to protect the entire cattle population (KNIGHT-JONES et al., 2014). Aluminium hydroxide gel mixed with saponin, and oil adjuvants are commonly used in inactivated FMD vaccines (AYELE et al., 2023). Oil adjuvant vaccines have been reported to have a superior immune response at any given time to aluminium hydroxide gel vaccines (PATIL et al., 2002). Therefore, oil adjuvant inactivated vaccines are currently used in Türkiye. To assess the effectiveness of vaccination regimens and programmes, post-vaccination serological monitoring is essential for countries implementing vaccine-based FMD control policies (KNIGHT-JONES et al., 2016; SINGH et al., 2019). Despite the previous studies that assessed the effectiveness of inactivated FMD vaccines (KNIGHT-JONES et al., 2014; KNIGHT-JONES et al., 2015; ÇOKÇALIŞKAN et al., 2016), the effectiveness of oil adjuvanted inactivated vaccines is not fully understood in Türkiye because KNIGHT-JONES et al. (2014) assessed the FMD Asia-1 inactivated vaccine's effectiveness in cattle in three provinces, and ÇOKÇALIŞKAN et al. (2016) investigated the effectiveness of the oil adjuvanted inactivated vaccine (containing O TUR_{07} and $ATUR_{11}$ strains) in only nine-month-old calves that were kept in closed containments. Therefore, this study aimed to evaluate protection levels after vaccination of cattle with an oil adjuvanted inactivated bivalent vaccine (containing A_{22} Iraq and O_1 Manisa FMDV strains) in a larger cattle population in the field.

Material and methods

Study area and sampling. This study was carried out in five provinces (Artvin, Rize, Trabzon, Giresun, and Ordu Provinces) in the Eastern Black Sea Region of Türkiye (Fig. 1). The major income sources for rural areas in all the provinces surveyed are cattle and fish farming. In these regions, the cattle industry is dominated by a substantial amount of

small (1-10 cattle) and medium-sized (11-50 cattle) family-run herds. Jersey and Turkish Brown Swiss breeds were prevalent, and there were 486,417 cattle in the provinces surveyed (TURKISH STATISTICAL INSTITUTE, 2011). The Anatolian region of Türkiye has an endemic FMD situation, and its control is based on the vaccination of cattle twice a year (KNIGHT-JONES et al., 2016; HERRERA-DIESTRA et al. 2022). The spread of FMDV in the population is deemed to be prevented by vaccination of at least 80% of cattle (WOAH, 2018). Therefore, at least 80% of the cattle population is routinely vaccinated every six months in Türki- ye. The two vaccination seasons are autumn and spring.

The number of cattle needed for sampling was determined using the formula described by THRUSFIELD (2007), and it was determined that 210 cattle should be sampled using a 5.4% acceptable error, 80% expected vaccine coverage, and a 95% confidence level. The number to be sampled was divided by the number of provinces, and the number of cattle to be sampled from each province was determined. To obtain results that reflect typical field protection after vaccination, a list of herds with no FMD record for many years in the selected provinces was obtained from the provincial agriculture and forestry directorates of each province in the autumn of 2009. The Randomize tool in Microsoft Excel software (MICROSOFT, 2019) was used to randomly select participants from the list, and contact herd owners from the five provinces. The herd owners contacted (n=71) agreed to participate in the study. It has been reported that maternal antibodies persisted for as long as three months in calves (LALZAMPUIA et al., 2022). Therefore, due to the possibility of maternal immunity, only animals older than 3 months were included in this study. A total of 42 cattle were selected from each province. The Turkvet animal registration system was used to check the FMD vaccination histories of selected cattle. Selected cattle were vaccinated with a 2 ml dose of inactivated bivalent vaccine (containing A_{22} Iraq and O_1 Manisa FMDV strains) formulated in oil adjuvant, containing at least $3PD_{50}$ ((protective doses) per dose) (Şap Institute, Ankara, Türkiye). Blood samples were collected from cattle (n=210) on day 0 (pre-vaccination period) and 28 days after vaccination. After the blood samples were centrifuged at 2000 rpm for 10 minutes, the serum samples were placed into sterile cryovial tubes, and stored at -25°C until analysis.

Serological tests. A solid-phase competition ELISA (SPC ELISA) was used to assess the serum for SP antibodies for the vaccine serotypes. The SPC ELISA was carried out according to the

Fig. 1. The location of the provinces included in the study is indicated on the map of Türkiye in blue

instructions of MACKAY et al. (2001). The plates were covered with an appropriate concentration of rabbit anti-FMDV antiserum specific for O_1 Manisa and A_{22} Iraq FMDV strains, and incubated at 4°C for 24 hours. The plates were washed three times using phosphate-buffered saline with 0.1% Tween (PBST). Then, each well of the ELISA plates was filled with FMDV O and A serotype antigens that were obtained from the Şap Institute (Ankara, Türkiye), and then left incubated at 37°C for 60 minutes. Following washing, test serum and control serum (negative serum, weak positive antiserum, and strong positive antiserum), diluted with blocking buffer, were added, followed by the addition of serotype-specific guinea pig antiserum, resulting in a final serum dilution of 1/5. After incubating at 37°C for 60 minutes, the plates were washed three times with PBST. All the wells were filled with blocking buffer containing anti-guinea pig immunoglobulin, conjugated with horseradish peroxidase, and incubated at 37°C for 60 minutes. Substrate/chromogen (0.05% H_2O_2 + Ortho-phenylenediamine dihydrochloride) was added to each well after washing. After 15 minutes, the reaction was stopped by adding 1.25 M H_2SO_4, and then OD values were measured at 492 nm wavelength using a spectrophotometer (Epoch, BIO-TEK, USA). The percentage of inhibition was computed for each well using a computer program (Softmax Pro-312ex, Molecular Devices, USA). Serum samples were considered positive if they had ≥60% inhibition (titre ≥1:7.5 or 2.9 $\log_2$), whereas serum samples with a titre ≥ 1:15 (3.9 $\log_2$) were considered protective (MACKAY et al., 2001).

Statistical analysis. The association between vaccine-induced antibody levels and serotype and age was evaluated with Fisher's exact test. The relationship between vaccine-induced antibody levels and provinces was assessed by one-way ANOVA. The statistical significance was established using a p-value of 0.05. SPSS software (IBM, 2020) was used to perform the statistical analysis.

Results

SPC ELISA. Before vaccination, out of the 210 serum samples tested, 53 samples had no detectable antibodies against serotype A, whereas 62 samples were found to be seronegative against serotype O (P=0.381). In the remaining samples, serotype A and serotype O antibody titres varied between 2.3 $\log_2$ and 3.6 $\log_2$, and 2.3 $\log_2$ and 3.3 $\log_2$, respectively (P=0.736). After vaccination, out of 210 serum samples tested, 167 (79.5%) cattle had seroconverted to serotype A ($\log_2$ titre 3.8 to 5.6), whereas 159 (75.7%) cattle had neutralizing antibodies ($\log_2$ titre 3.5 to 5.6) against serotype O (P=0.412). There was a significant difference between the proportions of animals with protective titres ($\log_2$ titre ≥3.9) to serotype A (152, 72.4%) compared to serotype O (130, 61.9%) (P=0.029).

Serum samples from Giresun Province had higher antibody titres against serotype A (41/42, 97.6%) compared to the serum samples from other provinces (Table 1), whereas serum samples from Ordu Province had higher antibody titres against serotype O (37/42, 88.1%) than other provinces. Serum samples from Trabzon Province had the lowest antibody titres against both serotype

Table 1. SP antibody testing results by SPC ELISA based on province

		Serotype A			Serotype O		
Province	**n**	**Negative n (%)**	**Positive n (%)**	**Protective n (%)**	**Negative n (%)**	**Positive n (%)**	**Protective n (%)**
Ordu	42	5 (11.9)	5 (11.9)	32 (76.2)	5 (11.9)	3 (7.1)	34 (81)
Giresun	42	1 (2.4)	-	41 (97.6)	6 (14.3)	11 (26.2)	25 (59.5)
Trabzon	42	15 (35.7)	2 (4.8)	25 (59.5)	15 (35.7)	2 (4.8)	25 (59.5)
Rize	42	13 (31)	3 (7.1)	26 (61.9)	13 (31)	4 (9.5)	25 (59.5)
Artvin	42	9 (21.4)	5 (11.9)	28 (66.7)	12 (28.6)	9 (21.4)	21 (50)
Total	210	43 (20.5)	15 (7.1)	152 (72.4)	51 (24.3)	29 (13.8)	130 (61.9)

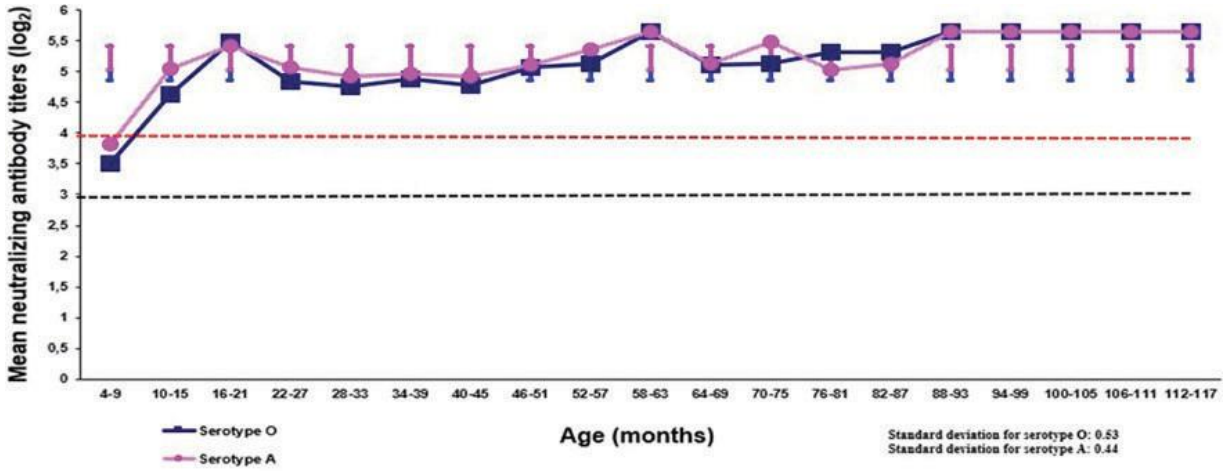

Fig. 2. Age-related changes in mean neutralizing antibody titres against serotypes O and A
The 95% confidence intervals are represented by error bars, black and red dashed lines indicate positive and protective titres, respectively

A (27/42, 64.3%) and serotype O (27/42, 64.3%) (P=0.022 for serotype A, P=0.042 for serotype O).

Mean neutralizing antibody titres for both serotype O (log_2 titre 5.0) and A (log_2 titre 5.1) were significantly higher in animals over 12 months than in animals between 4-11 months (log_2 titre 3.5 for serotype O, and log_2 titre 4.1 for serotype A) (P<0.001 for serotypes A and O). The age-related changes in mean neutralizing antibody titres are shown in detail in Fig. 2. SPC ELISA revealed that antibody titres against serotypes O and A were consistently elevated between 4 months to 21 months of age. Antibody titres levels decreased after 21 months of age before increasing again, but were still positive. This was statistically not significant (P>0.05) (Fig. 2).

Discussion

Vaccination is a reliable method for preventing clinical FMD and has been proven to be a highly effective strategy for preventing FMD outbreaks. The ability of FMD vaccines to control the disease is closely related to their ability to stimulate the formation of protective antibodies against the specific field strain (PARIDA, 2009). Immunological protection against FMD correlates with the induction of high levels of neutralizing antibodies in serum, which neutralize virus infectivity (JAIN et al., 2022). Vaccine-induced protective antibodies can be assessed accurately by measuring FMDV-neutralizing antibodies using serological assays (MANSILLA et al., 2020). WOAH recommends using SPC ELISA as a serological method to detect antibodies against FMDV (WOAH, 2022). Therefore, in this study, immune response to FMD vaccination in cattle was determined by SPC ELISA, which is a prescribed test for routine serological screening.

In this study, it was determined that sampled animals had lower pre-vaccination antibody lev- els for serotype A and serotype O, and there was a statistically significant difference in serotype A and serotype O antibody titres before and after vaccination (P=0.001). Additionally, the number of serotypes A and O seronegative cattle decreased after vaccination (P=0.296 for serotype A, P=0.271 for serotype O). This result is in agreement with a previous study suggesting antibody titre decreased 6 months after vaccination (ROCHA et al., 1983). This result can be explained by the timing of the vaccination. The farm owners reported that the animals were vaccinated against FMD 7-8 months previously. KNIGHT-JONES et al. (2015) reported that vaccinated cattle are likely to be susceptible to FMD serotypes A, O, and Asia-1 three to four

months after vaccination with inactivated vaccines. Therefore, it is recommended that cattle should be vaccinated every 4 to 6 months (DOEL, 2003; KNIGHT-JONES et al., 2015).

In this study, it was found that the rate of vaccine-induced antibody level against serotype A (79.5%) was higher than serotype O (75.7%) 28 days after vaccination. However, vaccine-induced antibody levels were not significantly different between serotype A and serotype O (P=0.412). Similarly, EL-BAGOURY et al. (2013) reported higher antibody titres against serotype A than serotype O in cattle vaccinated with oil-adjuvanted bivalent vaccine (containing O /3/93, A/Egypt/2006 FMDV strains). Furthermore, in this study the rate of antibodies above the protection level against serotype A (72.4%) was significantly higher than serotype O (%61.9) (P=0.029). This finding is consistent with previous studies (GUBBINS et al., 2022; WOLDEMARIYAM et al., 2023b). There are multiple reasons for this situation, including the 146 S particle disintegration of the virus when antigens are prepared for vaccine production, and the structural integrity of the intact virion, since serotype O is typically unstable compared to serotype A (KNIGHT-JONES et al., 2015). Even though serum reactivity to different viral strains may vary depending on the likelihood of protection, we cannot currently quantify this variation with confidence (PAY, 1984). To quantify protection, a virus challenge is necessary, either in the field or under controlled conditions. Correlation of protection with a specific assay is necessary for serological predictions. Furthermore, it has been noted that protection against serotype O requires higher antibody titres compared to protection against serotypes A and C (PAY and HINGLEY, 1987; VAN MAANEN and TERPSTRA, 1989).

In this study, vaccine-induced antibody levels against serotype A (27/42, 64.3%) and serotype O (27/42, 64.3%) were significantly lower in Trabzon Province than in other provinces. This difference in vaccine-induced antibody levels in different provinces can be explained by the vaccine potency, the vaccine administration route, the storage conditions of the vaccine before use, the host immune response, and the variations in management conditions.

The finding of this research is in agreement with EL-BAGOURY et al. (2013) who reported that the protective neutralizing serum antibody titre started within the first month of vaccination (Fig. 2). Furthermore, animals over 12 months had significantly higher mean neutralizing antibody titres for both serotypes O and A compared to animals between 4-11 months (P<0.001 for serotypes A and O). This finding is in agreement with previous studies (ELNEKAVE et al., 2016; LALZAMPUIA et al., 2022). LALZAMPUIA et al. (2022) reported that vaccine-induced antibody levels were higher in older animals (>2 years) than in animals less than two years old. However, the finding of this study disagrees with a previous report that young buffaloes had higher antibody titres than older animals (MOHAN et al., 2014). The reason for this may be the variations in experiment setup, vaccines used, inoculation route, and the number of experimental animals.

In this study, the immune response trend was observed to increase between 4 and 21 months of age (Fig. 2). However, after 21 months of age, serum-neutralizing antibody titres for the serotypes O and A slightly decreased from 5.48 $\log_2$ to 4.84 $\log_2$ and 5.42 $\log_2$ to 5.07 $\log_2$, respectively. Then, an increase in serum neutralizing antibody titres was observed again from the age of 28-33 months (Fig. 2). There are multiple reasons for this situation, including the quality of FMD vaccines, host immune response, and the number of vaccinations. In this study, according to the vaccination records, it was determined that the first three vaccinations were administered between the ages of 4 and 21 months, and the fourth vaccination was administered between the ages of 22 and 27 months. It has been reported that the administration of the fourth vaccination resulted in a consistent protective antibody titre for a long period (ELNEKAVE et al., 2016). In this study, after the fourth vaccination, serum antibody titres for the serotypes A and O increased again and remained high (Fig. 2).

There are some limitations to this study. First, due to budgetary limitations, we were unable to compare the effectiveness of available vaccines in a larger sample size. Second, due to the study being conducted in the Eastern Black Sea region

of Türkiye, there are concerns about geographical variation, and the generalisability of the results to all regions of Türkiye is uncertain.

Conclusions

The results of this study revealed that the inactivated bivalent vaccine (containing O_1 Manisa and A_{22} Iraq FMDV strains) provided a reasonable level of protection against serotypes A and O. However, antibodies above the protection level against serotype O (61.9%) were lower than the protection level recommended by WOAH. WOAH recommends that FMD vaccines provide a protective titre of 70% of the vaccinated population (WOAH, 2022). Furthermore, vaccine performance varied from province to province. Field conditions, coverage, husbandry, vaccine potency, and delivery (such as cold chain or shelf life adherence) can affect vaccine performance. Therefore, the effectiveness of the vaccine program should be evaluated through post-vaccination monitoring.

Ethical approval

This study was approved by the Animal Eth- ics Committee at the Selçuk University (Approval number: 2008/081).

Declaration of competing interest

The author declares that there is no conflict of interest.

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ŞEVIK, M., F. F. ÖZTÜRK: Imunosni odgovor goveda protutijelima na inaktivirano bivalentno cjepivo s uljnim adjuvansom za virus slinavke i šapa. Vet. arhiv 96, 11-20, 2026.

SAŽETAK

Slinavka i šap (FMD) važna je virusna bolest papkara, rasprostranjena u cijelom svijetu, na koju se može utjecati raznim strategijama, uključujući i cijepljenje. Cilj je istraživanja bio procijeniti imunosni odgovor protutijelima u goveda koja su cijepljena protiv slinavke i šapa. Ukupno je 210 jedinki iz različitih stada (n=71) u Crnomorskoj regiji Turske cijepljeno inaktiviranim bivalentnim cjepivom s uljnim adjuvansom, koje je sadržavalo sojeve virusa slinavke i šapa A_{22} Iraq i O_1 Manisa. Uzorci seruma prikupljeni su 28 dana nakon cijepljenja. Protutijela proizvedena u cijepljenih goveda mjerena su testom ELISA s antigenom na površini nosača čvrste faze. Prije cijepljenja, u 53 uzorka nije se mogao otkriti serotip A, dok su 62 uzorka bila seronegativna na serotip O (P=0,381). U preostalim je uzorcima titar protutijela na serotip A varirao između 2,3 $\log_2$ i 3,6 $\log_2$, a na serotip O između 2,3 $\log_2$ i 3,3 $\log_2$. Ukupno je 167 (79,5%) od 210 cijepljenih goveda imalo serokonverziju u serotip A (titar $\log_2$ 3,8 u 5,6), dok je 159 jedinki (75,7%) imalo neutralizacijska protutijela (titar $\log_2$ 3,5 u 5,6) na serotip O. Uočena je statistički znakovita razlika u titrima protutijela serotipova A i O prije i poslije cijepljenja (P=0,001). Također, poslije cijepljenja udio životinja sa zaštitnim titrom ($\log_2 \geq 3,9$) znakovito se razlikovao između serotipa A (152; 72,4%) i serotipa O (130; 61,9%) (P=0,029). U usporedbi sa životinjama starosti 4 – 11 mjeseci, životinje starije od 12 mjeseci imale su znakovito veću srednju vrijednost titra neutralizacijskih protutijela za oba serotipa, O i A (P=0,001). Rezultati istraživanja otkrili su da inaktivirano bivalentno cjepivo pruža dostatnu razinu zaštite protiv serotipa A i serotipa O. Međutim, protutijela čije su vrijednosti bile iznad zaštitne razine za serotip O imala su relativno male vrijednosti u cijepljenih goveda u usporedbi sa serotipom A. Zaključuje se da bi u procjeni učinkovitosti cjepiva protiv virusa slinavke i šapa trebalo provoditi daljnje praćenje goveda nakon cijepljenja.

Ključne riječi: slinavka i šap; titar neutralizacijskih protutijela; praćenje nakon cijepljenja; serotip A; serotip O; cjepivo