

Original research article

## **DECLINE OF MATERNAL ANTIBODIES AGAINST SWINE INFLUENZA A AND PRRS IN PIGLETS FROM DIFFERENT SOW VACCINATION PROGRAMS**

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### **Abstract**

The primary objective of this study was to evaluate the effect of sow influenza vaccination timing on the persistence and decay of maternally derived antibodies (MDAs) against swine influenza A virus (IAV-S) in piglets. PRRSV antibody dynamics were additionally assessed to provide contextual information on maternally derived antibody decline and post-vaccination seroconversion under the same herd conditions. A total of 200 piglets (20 per group at each of five time points: days 5, 28, 42, 56, and 70) were sampled in a cross-sectional design. Serum antibodies against IAV-S and PRRSV were measured using commercial ELISA assays. Piglets born to sows vaccinated against IAV-S three weeks before farrowing (Group A) exhibited higher and more consistent MDA titers compared to those from sows vaccinated four months earlier (Group B). At day 56, 75% of piglets in Group A and 60% in Group B remained seropositive for IAV-S. By day 70, seropositivity declined to approximately 40–45% in both groups. A marked decline in antibody levels was observed over time, with a significant proportion of piglets becoming seronegative by eight weeks of age. For PRRSV, antibody levels declined until day 42 and increased thereafter following piglet vaccination,

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indicating successful induction of active immunity. These results highlight the critical role of sow vaccination timing in shaping MDA kinetics and the need to align piglet vaccination strategies accordingly. Furthermore, they emphasize the necessity of integrated control approaches, beyond vaccination alone, to effectively manage PRRSV and IAV-S transmission in endemically infected herds.

**Key words:** swine, porcine reproductive and respiratory syndrome virus, swine influenza, maternal antibodies, vaccination

## INTRODUCTION

Swine Influenza and Porcine Reproductive and Respiratory Syndrome (PRRS) are among the most significant infectious diseases in modern swine production (Saade et al., 2020). Both are viral diseases caused by RNA viruses, which are characterized by high mutation rates. As such, continuous monitoring and adaptation of control programs are essential. Swine Influenza is most commonly caused by three subtypes of Influenza A virus (IAV): H1N1, H1N2, and H3N2, originating from human, avian, or swine sources (Ma, 2020). Pigs are considered mixing vessels for influenza viruses, facilitating the emergence of new strains with pandemic potential (Ma et al., 2009). PRRS virus (PRRSV) is highly diverse but generally classified into two species: *Betaarterivirus suid 1* (PRRS-1) and *Betaarterivirus suid 2* (PRRSV-2) (Taira et al., 2025). Ongoing viral evolution and genetic diversification continue to result in the emergence of novel variants, some of which are associated with increased pathogenicity. (Li et al., 2024). PRRSV infections are especially significant in neonatal and early post-weaning pigs because their adaptive immune systems are not fully developed and they depend on maternally derived antibodies during a critical window of immunological development (Butler et al., 2014). In contrast, endemic swine influenza A virus (IAV-S) circulation within herds is maintained by continuous introduction of susceptible hosts, and as maternal antibodies decline in the nursery and early grower stages, stress and increased exposure contribute to disease susceptibility (Schmies et al., 2024). Additionally, PRRS causes reproductive disorders in sows and impairs semen quality in boars (Schulze et al., 2013). Following IAV-S infection, the pigs mount a humoral immune response with the production of specific antibodies (Salvesen and Whitelaw, 2021). However, this immunity is short-lived, often necessitating repeated vaccination (Petro-Turnquist et al., 2025). In contrast, PRRSV rather modulates the cellular immune response, although antibody levels are still used as indicators of infection and immune status (Toman et al., 2019). IAV-S infection typically leads to efficient viral clearance, whereas PRRS infection is characterized by prolonged persistence and incomplete viral elimination (Toman et al., 2019). Vaccination against both diseases is widely used, with vaccines tailored to match circulating strains for optimal efficacy. Although vaccination is a valuable tool for disease control, it does not completely prevent viral circulation or the emergence of new strains (Ma,

2020; Li et al., 2024). Therefore, vaccination protocols must be carefully designed and regularly updated. In early life, piglets are protected by maternally derived antibodies (MDA), whose high levels can neutralize vaccine antigens and block immune response, a phenomenon known as maternal antibody interference. Thus, the optimal time for vaccination is when MDA levels decline below the threshold of detection, allowing the piglet's immune system to respond effectively. The primary aim of this study was to compare maternally derived antibody kinetics against IAV-S in piglets born to sows vaccinated either three weeks or four months prior to farrowing. Additionally, PRRSV antibody dynamics were evaluated under a uniform sow vaccination schedule. Although IAV-S and PRRSV differ substantially in pathogenesis and immune response, both infections are endemic respiratory pathogens commonly controlled through sow and piglet vaccination programs in commercial swine production systems. The inclusion of PRRSV in this study was not intended as a direct comparison with IAV-S pathogenesis, but rather to provide contextual insight into maternally derived antibody decline and post-vaccination serological responses within the same herd environment under standardized management conditions.

## **MATERIALS AND METHODS**

### ***Animals and experimental design***

All procedures were approved by the Ethics Committee of the Institute of Veterinary Medicine of Serbia under approval No. EK-2025/M06 and were conducted in accordance with national animal welfare regulations. The study was conducted on a commercial farrow-to-nursery farm with approximately 2000 sows operating a batch-farrowing system of 300 sows per batch. The farm operated under endemic vaccination protocols for IAV-S and PRRSV and no clinical outbreaks were observed during the study period. Two groups of piglets born to sows with different influenza vaccination timings were included: Group A sows were vaccinated with RespiPorc Flu3 (Ceva Santé Animale), an inactivated trivalent swine influenza vaccine containing H1N1, H1N2, and H3N2 strains, administered three weeks prior to farrowing. Group B sows received the same vaccine four months before farrowing. All sows were vaccinated against PRRSV using ReproCyc PRRS EU (Boehringer Ingelheim), a modified live PRRSV-1 vaccine, three weeks prior to farrowing. Piglets were vaccinated at weaning (day 21) with PRRS FLEX EU (Boehringer Ingelheim), a modified live PRRSV-1 vaccine. The study involved sows of parities 1–5, representing a typical mid-parity distribution for commercial herds, and all were selected from the same production batch to minimise batch-related variability. This study followed a cross-sectional design. A cross-sectional approach was chosen to reflect herd-level immunity dynamics under commercial production conditions. Individual piglets were not followed longitudinally

throughout the study period. Repeated sampling of the same piglets was avoided to minimize handling stress and potential immune modulation. Limiting inclusion to a maximum of two piglets per litter reduced litter-related bias while enabling population-level estimation of MDA distribution. At each sampling point (days 5, 28, 42, 56, and 70), twenty different piglets per group were randomly selected, with no re-sampling of individuals across time points. A maximum of two piglets per litter was included at each sampling to avoid overrepresentation of individual sows. In total, 200 piglets were sampled throughout the study (20 piglets  $\times$  2 groups  $\times$  5 sampling points). The analytical approach differed between IAV-S and PRRSV due to differences in sow vaccination schedules. For IAV-S, two distinct vaccination intervals were compared, whereas for PRRSV all sows followed a standardized schedule, allowing evaluation of MDA decline and post-vaccination seroconversion without between-group comparison.

### ***Sample collection***

Blood samples were collected from piglets in both groups on days 5, 28, 42, 56, and 70 post-farrowing. Serum was separated by centrifugation and stored at  $-20^{\circ}\text{C}$  until analysis. Nasal swabs were collected from a subset of 20 piglets per time point, primarily those exhibiting clinical signs, to monitor potential field IAV and PRRSV circulation at the herd level. Nasal samples were obtained using sterile cotton swabs inserted into both nostrils and rotated to ensure adequate mucus collection. Samples were immediately chilled, transported to the laboratory within the same day, and stored at  $-80^{\circ}\text{C}$  until nucleic acid extraction and PCR analysis.

### ***Serological testing***

Serum samples were tested for the presence of antibodies against PRRSV and IAV-S using commercial enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturers' instructions. Detection of PRRSV antibodies was performed using the PrioCHECK<sup>®</sup> PRRSV Antibody ELISA Kit (Thermo Fisher Scientific). For Influenza A virus antibodies, the INGEZIM Influenza A ELISA kit (Ingenasa) was used. Samples with competition percentages (%C)  $\leq 30$  were considered seropositive, in accordance with the manufacturer's recommended cut-off value. The PrioCHECK<sup>®</sup> PRRSV Antibody ELISA detects antibodies directed against PRRSV nucleocapsid (N) protein antigens. The INgezim Influenza A ELISA detects antibodies against conserved Influenza A nucleoprotein (NP) antigens, enabling broad detection of antibodies against multiple IAV-S subtypes.

## Molecular testing

RNA was extracted from nasal swabs immersed in 1 mL phosphate-buffered saline (PBS), vortexed, and centrifuged at 2000 g for 10 min using the IndiSpin Pathogen Kit (Indical Bioscience, Germany). For molecular detection, real-time reverse transcription quantitative PCR (RT-qPCR) assays targeted the the ORF7 region of PRRSV and matrix (M) gene of Influenza A virus using the Luna® Universal Probe One-Step Kit (NEB, USA) according to previously published protocols (Opriessnig et al., 2006, Shu et al., 2011). IAV testing was done at all sampling points, while PRRSV testing was limited to pre-vaccination samples due to possible detection of the live vaccine strain.

## Statistical analysis

Data were analyzed using a two-way ANOVA to assess the effects of sampling time and vaccination group on ELISA antibody reactivity, including their interaction (Time  $\times$  Vaccination). As different piglets were sampled at each time point, a between-subjects design was applied. Homogeneity of variance was tested with Levene's test. For PRRSV, only sampling time was analyzed using one-way ANOVA, since all piglets came from vaccinated sows. Analyses were performed in Numiqo online application (<https://numiqo.com/>) with significance set at  $P < .05$ .

## RESULTS

Most animals in both groups remained seropositive up to the fourth sampling point (day 56), with 75% of piglets in Group A (Figure 1) and 60% in Group B (Figure 2) testing positive.

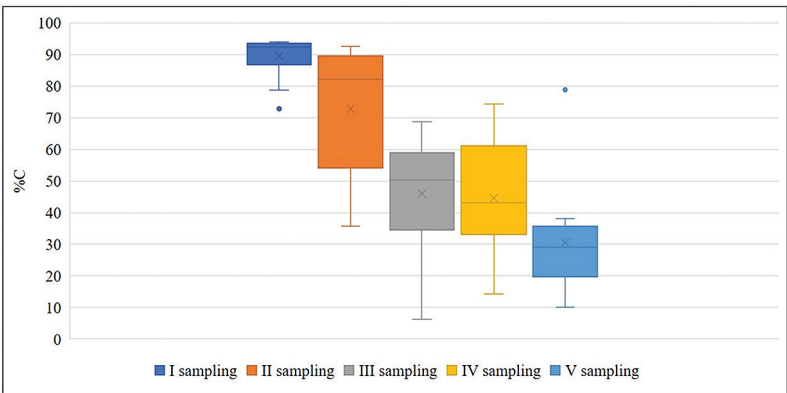


**Figure 1.** Distribution of %C values for IAV-S antibodies in Group A piglets across five sampling points. The data represent all tested piglets, including both seropositive and seronegative animals

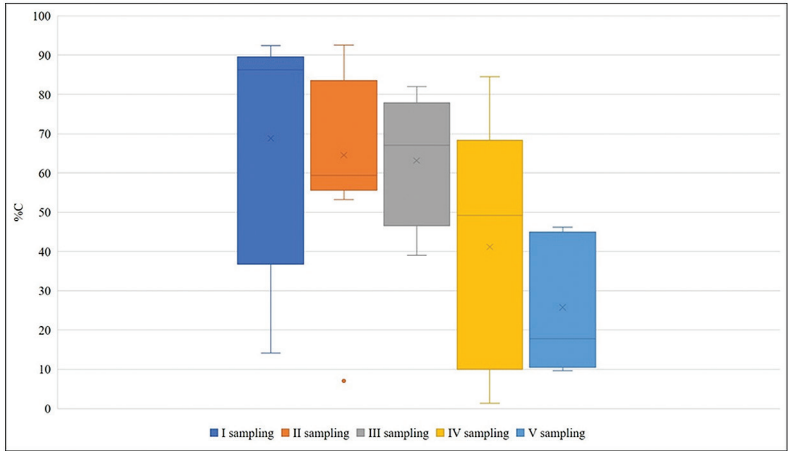


**Figure 2.** Distribution of %C values for IAV-S antibodies in Group B piglets across five sampling points. The data include all tested piglets, incorporating both seropositive and seronegative animals

A significant variance difference was observed at the first sampling ( $P = .018$ ), with Group A showing more consistent values and Group B greater variability. No variance differences were detected thereafter. By day 70, most piglets in both groups were seronegative; Group B's mean % competition fell below 30%, while Group A remained slightly higher (Table 1). Two-way ANOVA showed a significant effect of sampling time on antibody levels ( $F(4, 64) = 19.98, P < .001$ ), but no significant effects of vaccination group ( $F(1, 16) = 0.12, P = 0.739$ ) or interaction ( $F(4, 64) = 0.12, P = 0.054$ ), indicating similar antibody decline patterns between groups.

Table 1: Percentage of seropositive piglets and mean competition percentage (%C) for IAV-S antibodies in Group A and Group B across five sampling points

|                           | Group A days post-farrowing |      |      |      |       | Group B days post-farrowing |      |      |      |      |
|---------------------------|-----------------------------|------|------|------|-------|-----------------------------|------|------|------|------|
|                           | 5                           | 28   | 42   | 56   | 70    | 5                           | 28   | 42   | 56   | 70   |
| % of seropositive piglets | 100                         | 100  | 80   | 75   | 45    | 80                          | 95   | 100  | 60   | 40   |
| % C                       | 89.5                        | 72.8 | 46.1 | 44.6 | 30.46 | 68.8                        | 64.5 | 63.2 | 40.6 | 25.7 |

Regarding PRRSV, MDA levels, reflected by seropositivity rates and percentage positivity (PP) values, declined until day 42. Following vaccination of piglets at day 21, PP values subsequently increased, indicating seroconversion and the development of active humoral immunity. By day 70, nearly all piglets were seropositive with high antibody levels. The results showed no significant effect of sampling time ( $F(4, 40) = 2.37, P = 0.069$ ), though trends indicated waning maternal antibodies followed by a post-vaccination rise. All nasal swabs tested negative for IAV-S and PRRSV, confirming absence of viral circulation or shedding.

## DISCUSSION

Vaccination against PRRSV and IAV-S is routinely implemented in modern swine production systems. The standard PRRSV vaccination protocol involves immunizing piglets at one day of age if born to seronegative sows, or around the time of weaning if born to seropositive sows (Balasch et al., 2018). Gilts and sows are typically vaccinated every 3–4 months and again approximately three weeks prior to breeding. The study was conducted in a commercially managed herd with endemic vaccination against IAV-S and PRRSV and no evidence of active viral circulation during the sampling period, as confirmed by negative PCR results. Therefore, maternal antibody dynamics were evaluated under conditions of stable vaccination pressure rather than acute outbreak exposure.

Vaccination against IAV-S is performed multiple times per year. The primary objective of vaccinating breeding females is to enhance the level of maternal antibodies transferred to piglets, thereby providing early protection against IAV-S. The results of this study indicate that piglets born to sows vaccinated three weeks prior to farrowing exhibited more persistent maternally derived antibody responses against IAV-S, as reflected by ELISA %C values and seropositivity rates. This underscores the critical importance of optimal sow vaccination timing in enhancing passive immunity during the early life stages of piglets. Although MDA levels can vary, protective titers can be detected in piglets up to 14 weeks of age (Pyo et al., 2015), which is consistent with our findings, where 40–45% of piglets remained seropositive at 70 days of age. Despite the early protection conferred by MDAs, fewer than 80% of piglets remained protected (presumed protective threshold based on manufacturer cut-off values) at 8 weeks of age in our study, resulting in a critical period of susceptibility to IAV-S infection. Therefore, in endemic farms, it is essential to implement additional biosecurity measures, as intra-subtype variations of IAV-S have been shown to compromise cross-protection and may result in acute outbreaks. Moreover, infection with homologous strains in piglets possessing MDAs can lead to prolonged viral shedding and potentially facilitate the emergence of new viral variants (Ryt-Hansen et al., 2019). The presence of MDA at vaccination may also promote wild-type virus transmission, contributing to on-farm persistence (Andraud et al., 2023). While manufacturers recommend piglet vaccination at 56 days, this should be considered a minimum, as 60–75% of piglets in our study were still seropositive at that age. Maternal immunity has been shown to reduce viral titers in piglets challenged with homologous IAV-S strains, but not with heterologous ones (Keay et al., 2023). Once infection occurs, no significant difference in the duration of viral shedding was observed between MDA-positive and MDA-negative pigs (Keay et al., 2023). In our study, all nasal swabs tested negative for both IAV and PRRSV throughout the observation period, confirming the absence of viral shedding or field virus circulation. These results suggest that MDAs, in combination with vaccination and implemented biosecurity measures,

were associated with the absence of detectable viral circulation under the study conditions. However, as the level of infection pressure on the farm was not directly assessed, definitive conclusions regarding prevention of viral replication cannot be drawn (Schulze et al., 2013). While our study focused on herd-level protection, other studies emphasize that piglets with low MDA levels respond more similarly to MDA-negative individuals, underscoring the importance of analyzing individual-level data rather than relying solely on group means (Keay et al., 2023).

Similarly to IAV-S, vaccination of sows with modified live PRRSV vaccines results in the passive transfer of MDAs to piglets, providing transient protection that typically lasts from 2 to 11 weeks post-farrowing (Augustyniak and Pomorska-Mól, 2023). However, high MDA titers suppress early vaccine virus replication, resulting in no neutralizing antibodies detected until 14 weeks of age. In contrast piglets with low MDA titers exhibited earlier viral replication and a more robust cellular immune response, including increased numbers of interferon gamma IFN- $\gamma$ -secreting cells (Fablet et al., 2016). Similarly, it was reported (Renson et al., 2019) that at five weeks of age, only 44% of piglets with high MDA levels seroconverted following vaccination, compared to 94% in the low-MDA group. These findings are consistent with our observations, which show an initial decline in antibody levels, followed by a secondary increase, suggesting delayed seroconversion in the presence of MDAs. In our study, MDA levels peaked during the first four weeks of life and declined rapidly thereafter, reaching minimal titers between six and eight weeks. This decline may be further influenced by vaccination, as antibodies are consumed during antigen opsonization, rendering them undetectable by the ELISA method employed. However, given that effective herd immunity usually develops four to five weeks after vaccination, immunizing piglets at three weeks may serve as a practical compromise between early intervention and MDA interference. By ten weeks of age, all piglets in our study population had seroconverted, indicating successful induction of active immunity despite the initial presence of maternal antibodies. This study suggests that the timing of maternal vaccination plays a crucial role in determining both the duration and uniformity of antibody protection in piglets against IAV-S and PRRSV. The greater variability in initial IAV-S antibody levels observed in Group B piglets (born to sows vaccinated four months before farrowing) suggests that longer intervals between sow immunization and parturition may result in less consistent passive immunity transfer. In contrast, piglets from sows boosted three weeks pre-farrowing (Group A) exhibited more uniform antibody levels, underscoring the practical value of a pre-farrowing booster to achieve a more homogeneous maternal antibody profile and enhance early-life protection. These findings highlight the importance of optimizing sow vaccination schedules, together with appropriately timed piglet vaccination and strict biosecurity measures, for effective disease control. Although antibody measurements from sows at farrowing would have further strengthened the interpretation of maternal antibody transfer, such data were not available within this

study design. Antibody levels in piglets may vary substantially within litters due to differences in colostrum intake and individual immune response. Colostrum intake was not individually quantified in this field-based study. Although all piglets were managed under standardized commercial conditions with no evidence of impaired lactation, variation in colostrum consumption may contribute to individual differences in early MDA levels. The cross-sectional design and inclusion of piglets from multiple litters were intended to reduce litter-specific bias. However, residual variability related to passive transfer efficiency cannot be excluded. The observed heterogeneity in MDA levels further underlines the importance of herd-level monitoring and tailored management strategies to minimize protection gaps and reduce the risk of viral persistence and evolution in swine populations. While ELISA testing offered valuable insight into the temporal dynamics of humoral immunity, the obtained results represent relative measures of antibody reactivity rather than absolute concentrations. The %C and PP values provided by commercial ELISA kits reflect the strength of antigen–antibody interactions, allowing differentiation between seropositive and seronegative animals and comparison of antibody trends over time. However, these optical density–based indices are not linearly correlated with serum antibody concentration and should be interpreted as semi-quantitative indicators of immune status. Although the present findings describe relative changes in antibody reactivity associated with the decline of maternally derived antibodies and the onset of vaccine-induced responses, they do not quantify antibody concentration or neutralizing capacity. To better assess protective immunity, future studies should include quantitative or functional assays such as virus neutralization (VNT) or hemagglutination inhibition tests (HIT), along with cellular immune parameters like cytokine profiling to more accurately define the dynamics of maternal immunity. Unlike ELISA, which measures antibody reactivity, VNT and HIT quantify protective antibody titers and therefore provide a more direct assessment of the piglets' ability to neutralize circulating virus. Including such functional measures would help define meaningful correlates of protection, reduce uncertainty associated with interpreting %C values alone, and offer clearer insight into the true dynamics of maternally derived immunity. The present study was designed as a cross-sectional field investigation; therefore, individual piglets were not followed longitudinally throughout the sampling period. Consequently, the findings reflect herd-level trends in antibody dynamics rather than precise individual decay trajectories. While a longitudinal design with individually tagged piglets sampled from birth through the nursery phase would provide more accurate characterization of individual MDA kinetics, the cross-sectional approach employed here allowed robust estimation of population-level immunity patterns under commercial production conditions. Future studies incorporating longitudinal monitoring alongside functional antibody assays would further refine understanding of individual MDA decay and protective immunity development. Despite these limitations, this study provides robust field-based

evidence by comparing two practical sow vaccination intervals under standardized production conditions, offering directly applicable insights for refining vaccination schedules and strengthening herd-level immunity.

## CONCLUSION

This field-based study demonstrated that piglets born to sows vaccinated against IAV-S three weeks prior to farrowing exhibited more uniform and prolonged maternally derived antibody responses compared with piglets from sows vaccinated four months earlier. PRRSV antibody dynamics additionally illustrated the decline of maternally derived antibodies followed by post-vaccination seroconversion under commercial herd conditions. The findings suggest that sow vaccination timing may influence the duration and uniformity of passive immunity in piglets. However, the study was conducted in a single herd using a cross-sectional design and relatively limited sample size. Therefore, broader conclusions regarding optimal vaccination protocols and herd-level protection should be interpreted cautiously. Further longitudinal studies incorporating functional immunity assays and larger multi-herd populations are needed to better define protective antibody thresholds and optimal vaccination timing.

## Author's contribution

VM was responsible for the conceptualization and writing of the original draft. SŠ conducted the formal analysis. SS carried out the investigation. GR contributed to the development of the methodology. DR and SR provided resources. VM supervised the work. DG prepared the visualizations. DR contributed to the review and editing of the manuscript.

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## Ethical statement

All procedures were approved by the Ethics Committee of the Institute of Veterinary Medicine of Serbia under approval No. EK-2025/M06 and were conducted in accordance with national animal welfare regulations.

## Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

## Competing interests

One of the co-authors is an employee of Boehringer Ingelheim (BI). Boehringer Ingelheim had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. All other authors declare no conflicts of interest.

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## OPADANJE MATERNALNIH ANTITELA PROTIV INFLUENCE A SVINJA I PRRS-a KOD PRASADI KOD RAZLIČITIH VAKCINALNIH PROGRAMA KRMAČA

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### Kratak sadržaj

Primarni cilj ove studije bio je procena uticaja vremena vakcinacije krmača protiv virusa influenza svinja (IAV-S) na održavanje i opadanje titra maternalnih antitela (MDA) kod prasadi. Dinamika antitela protiv virusa respiratornog i reproduktivnog sindroma svinja (PRRSV) dodatno je ispitivana radi dobijanja kontekstualnih informacija o opadanju maternalnih antitela i serokonverziji nakon vakcinacije u istim uslovima zapata.

Ukupno 200 prasadi (20 po grupi u svakoj od pet vremenskih tačaka: 5., 28., 42., 56. i 70. dana života) uzorkovano je tokom istraživanja sprovedenog po principu presečnog ispitivanja. Antitela protiv IAV-S i PRRSV u serumima određivana su primenom komercijalnih ELISA testova. Prasad poreklom od krmača vakcinisanih protiv IAV-S tri nedelje pre prašenja (Grupa A) ispoljila su više i ujednačenije titre maternalnih antitela u poređenju sa prasadi poreklom od krmača vakcinisanih četiri meseca ranije (Grupa B). Pedeset šestog dana života, 75% prasadi u Grupi A i 60% prasadi u Grupi B ostalo je seropozitivno na IAV-S. Do 70. dana života seropozitivnost je opala na približno 40–45% u obe grupe. Tokom vremena zabeleženo je izraženo opadanje nivoa antitela, pri čemu je značajan procenat prasadi postao seronegativan do osme nedelje života. Kod PRRSV, nivo antitela opadao je do 42. dana života, nakon čega je usled vakcinacije prasadi došlo do njegovog porasta, što ukazuje na uspešnu indukciju aktivnog imuniteta. Ovi rezultati ukazuju na značajnu ulogu vremena vakcinacije krmača u oblikovanju kinetike maternalnih antitela, kao i na potrebu usklađivanja strategije vakcinacije prasadi sa tim promenama. Pored toga, rezultati naglašavaju neophodnost primene integrisanih mera kontrole, koje prevazilaze samu vakcinaciju, u cilju efikasnog suzbijanja transmisije PRRSV i IAV-S u endemski inficiranim zapatima.

**Ključne reči:** svinje, reproduktivni i respiratorni sindrom svinja, influenza svinja, maternalna antitela, vakcinacija