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Exploring Serum Copeptin and Hematological Profile: A Comparative Analysis after Intradermal versus Intramuscular Porcine Reproductive and Respiratory Syndrome Virus Vaccination in Piglets

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Simple Summary: The objective of this study was to investigate the impact of intradermal (ID) versus intramuscular (IM) vaccination with a porcine reproductive and respiratory syndrome virus (PRRSV)-modified live vaccine (MLV) in piglets on serum copeptin and hematological profile. The study included 104 suckling piglets (2 weeks of age) from a commercial farrow-to-finish PRRSV-positive pig farm. Blood samples were collected from piglets at 4, 7, and 10 weeks of age. Blood samples were used for the performance of the complete blood count and examination by PCR for PRRSV and by ELISA for copeptin. No significant differences in serum copeptin levels and the number of blood cell counts were noticed in the same group over time and among groups. In conclusion, it seems that the PRRSV vaccination does not affect the levels of the released copeptin.

Abstract: This study aimed to investigate the impact of intradermal (ID) and intramuscular (IM) vaccination with a porcine reproductive and respiratory syndrome virus (PRRSV)-modified live vaccine (MLV) in piglets on serum copeptin levels and hematological profile. This study included 104 suckling piglets (2 weeks of age) from a commercial farrow-to-finish pig farm suffering from positive unstable PRRSV status. Animals were assigned to four groups, with two replicates (13 piglets/group/replicate); group A: IM vaccination with a PRRSV MLV vaccine, group B: ID vaccination with the same vaccine, group C: ID of Diluvac Forte, and group D: IM of Diluvac Forte. Blood samples were collected from the same three pigs/group/replicate at 4, 7, and 10 weeks of age. Blood samples were used for the performance of the complete blood count, and they were also examined by PCR for PRRSV and by ELISA for copeptin. No significant differences in serum copeptin levels and the number of blood cell counts (packed cell volume—PCV, numbers of white blood cells—WBCs, and platelets number—PLTs) were noticed in the same group over time and among groups. In conclusion, it seems that the vaccination against PRRSV does not affect the levels of the released copeptin. Based on our results, the measurement of serum copeptin could not be proposed as a potential stress biomarker in pigs.

Keywords: blood cell counts; copeptin; intradermal; serum; pig; PRRSV; vaccine

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1. Introduction

The precursor protein of pre-provasopressin contains vasopressin neurophysin II and copeptin. Copeptin is a peptide composed of 39 amino acids that comprise the C-terminal part of the arginine vasopressin (AVP) precursor that was found to be a stable and sensitive surrogate marker for AVP release [1–3]. All these proteins transfer from the hypothalamus to the posterior pituitary gland, and more specifically, copeptin and neurophysin II carry the AVP. They are stored and co-secreted in response to various stimuli, including osmotic changes, stress, and inflammation [4]. AVP plays a crucial role in osmolarity, homeostasis, and endocrinology and has hemodynamic and central nervous effects [5]. More specifically, AVP helps regulate the body's water balance by increasing water reabsorption in the kidneys, which reduces urine output and helps maintain proper hydration levels [6]. In addition to its role in water balance, AVP acts as a potent vasoconstrictor. This can help increase blood pressure and maintain adequate blood flow to vital organs, especially when blood pressure drops, such as during a hemorrhage or shock [7]. Furthermore, AVP is involved in the body's stress response system. It is released in response to stressors, such as physical injury, pain, or psychological stress, and helps modulate the body's response to these stressors [8]. The major problem with the detection of vasopressin's concentration is that its measurement is difficult and has low accuracy [9].

Unlike AVP, copeptin is stable for several days after blood withdrawal [10]; it is found in higher concentrations in blood because it is unbound to blood platelets [11], its detection does not require extraction processes or other complex pre-analytical steps [12], and it can be easily measured with a chemiluminescence test using a minimal volume of the biological sample [1–3,13]. So, copeptin is a good biomarker because it is easier to measure in blood samples and remains relatively stable even after prolonged storage. Moreover, copeptin degrades slowly in humans, showing that it may not have any significant function in circulation [11]. Furthermore, copeptin follows the amount of AVP in circulation [2,14]. For all the above reasons, copeptin is a surrogate marker for AVP, even though its role is still unknown [9].

In clinical practice, copeptin measurement is often used to aid in diagnosing, prognosis, and managing the mentioned conditions, particularly those related to water balance and stress responses [1,13]. It can provide valuable information about the body's response to various stressors and pathological states, helping clinicians in decision making and treatment strategies [15]. Previous studies in porcine experimental models (miniature pigs) evaluated the release kinetics of copeptin in acute myocardial infarction [16,17]. Recently, the measurement of salivary copeptin levels has been proposed as a potential non-invasive biomarker of anxiety in dogs [18].

Furthermore, copeptin, as a surrogate marker of AVP, is involved in water retention and vascular tone regulation, as well as in erythropoiesis, thrombocyte activity, and inflammation [19]. More specifically, the decrease in the AVP concentration may affect the packet cell volume (PCV) [20] or it may not [21]. Additionally, AVP seems to be involved in inflammation modulation [22], while it plays a pivotal role in hemostasis by activating multiple coagulation factors and facilitating thrombocyte aggregation [23].

Vaccination remains the most crucial and cost-effective preventive tool in the fight to limit porcine reproductive and respiratory syndrome virus (PRRSV) infection and its consequences [24,25]. Important evidence was presented in earlier studies to demonstrate that the degree of virological protection obtained from an intradermal (ID) vaccination with the PRRSV MLV vaccine remains unchanged when compared to an intramuscular (IM) vaccination [26,27]. In our earlier research, after administering a PRRSV-modified live vaccine (MLV) intramuscularly (IM) and intradermally (ID) to 7-week-old piglets, we observed notable variations in the piglets' serum angiotensin II (Ang II) [28]. Regarding its relationship with vaccinations, copeptin levels may depend on the vaccine, individual response, and underlying health conditions. Research in this area is ongoing, and further studies are needed to fully understand the implications of copeptin in the context of vaccinations.

This study aimed to investigate the impact of ID and IM vaccination with a PRRSV MLV vaccine in piglets on the serum copeptin levels, as well as the effect of the vaccination on hematological profile.

2. Results

2.1. PCR Testing

PRRSV infection was verified before the onset of the trial by qRT-PCR in blood serum samples. Every sampled animal was qRT-PCR-positive for PRRSV. Analysis of the full-length ORF5 sequence of the wild-type PRRSV strain isolated in the trial farm at that time showed a 90.7% nucleotide sequence identity with the DV strain (the data are available in Figure S1 of the Supplementary File). This strain is included in the commercial PRRSV MLV used in the current experiment.

According to the findings of qRT-PCR in serum samples from pigs belonging to all groups at 4, 7, and 10 weeks of age, pigs of all the experimental groups were negative at the age of 4 weeks. All pigs were positive at the age of 10 weeks due to natural infection (the data are available in Table S1 of the Supplementary File). At the age of 7 weeks, all the pigs of group A were negative, while 33.33% of the pigs of group B [Ct: 35.2 (32.3–38)] and 83.33% of the pigs of groups C [Ct: 34.7 (25.5–40.8)] and D [Ct: 29.2 (24.2–32.2)] were positive. However, the Ct value of qRT-PCR seems to be lower in group A [33.3 (30.5–36.5)] compared to that of group B [34.6 (29.5–39.7)] at the age of 10 weeks.

2.2. Copeptin Results

The results for the serum copeptin levels are shown in Table 1. No significant differences were noticed in the same group over time, as well as among groups.

Table 1. Mean and standard deviation (SD) of copeptin levels in serum samples at ages of 4, 7, and 10 weeks.

Group	Time (Weeks)	Copeptin Levels (Mean ± SD)	Significant Differences in the Same Group over Time	Significant Differences among Groups
Group A (Porcilis PRRS ID)	4 weeks	88.10 ± 11.66	ns	4, 7, 10 weeks ns
	7 weeks	70.79 ± 35.21		
	10 weeks	93.56 ± 51.45		
Group B (Porcilis PRRS IM)	4 weeks	74.95 ± 23.17	ns	
	7 weeks	87.63 ± 29.76		
	10 weeks	67.33 ± 40.30		
Group C (Diluvac ID)	4 weeks	64.44 ± 21.77	ns	
	7 weeks	78.97 ± 28.53		
	10 weeks	93.22 ± 32.37		
Group D (Diluvac IM)	4 weeks	78.71 ± 27.39	ns	
	7 weeks	67.17 ± 24.07		
	10 weeks	79.51 ± 40.65		

ns: no significant differences.

2.3. Correlation between PRRSV Viral Load and Copeptin

No significant correlation was noticed either in the total number of animals at both time points or separately at each time point.

2.4. Hematological Results

The results for the number of blood cells are shown in Tables 2 and 3. There were no significant differences in the same group or among groups.

Table 2. Mean and standard deviation (SD) of packed cell volume (PCV), white blood cells (WBCs), and platelet number (PLT) at ages of 4, 7, and 10 weeks.

Group	Time (Weeks)	PCV (%) (Mean ± SD)	WBC (Mean ± SD)	PLT (Mean ± SD)	Significant Differences in the Same Group over Time	Significant Differences among Groups
Group A (Porcilis PRRS ID)	4	35.52 ± 3.66	10,976.92 ± 3596.33	2,555,384.62 ± 118,145.02	ns	4, 7, 10 weeks ns
	7	35.87 ± 3.96	14,784.61 ± 4855.38	296,286.15 ± 136,115.35		
	10	35.76 ± 3.62	12,584.61 ± 4141.55	237,415.38 ± 100,738.75		
Group B (Porcilis PRRS IM)	4	33.95 ± 4.15	12,247.69 ± 4018.61	278,369.23 ± 86,354.68	ns	
	7	34.51 ± 3.73	16,469.23 ± 5508.38	222,695.38 ± 69,083.74		
	10	33.43 ± 3.66	14,330.77 ± 4704.14	275,300.00 ± 98,715.60		
Group C (Diluvac ID)	4	35.33 ± 4.59	11,815.38 ± 3874.02	277,861.54 ± 142,872.41	ns	
	7	35.31 ± 4.65	13,553.84 ± 4446.64	246,946.15 ± 93,303.90		
	10	35.78 ± 4.88	14,184.61 ± 4654.72	31,115.15 ± 117,562.92		
Group D (Diluvac IM)	4	34.39 ± 4.68	12,061.54 ± 3642.24	334,553.85 ± 154,769.98	ns	
	7	34.42 ± 4.11	13,407.69 ± 3264.06	299,123.08 ± 83,916.32		
	10	34.09 ± 4.41	14,130.77 ± 2109.26	296,923.08 ± 130,245.94		

ns: no significant differences.

Table 3. Mean and standard deviation (SD) of neutrophils (Neu), lymphocytes (Lymph), monocytes (Mono), and eosinophils (Eos) at ages of 4, 7, and 10 weeks.

Group	Time (Weeks)	Neu (Mean ± SD)	Lymph (Mean ± SD)	Mono (Mean ± SD)	Eos (Mean ± SD)	Significant Differences in the Same Group over Time	Significant Differences among Groups
Group A (Porcilis PRRS ID)	4	6611.00 ± 1972.24	3363.77 ± 1508.11	609.07 ± 371.85	393.07 ± 283.80	ns	4, 7, 10 weeks ns
	7	8881.65 ± 2917.80	4986.69 ± 1793.29	532.73 ± 265.04	417.77 ± 205.51		
	10	7680.40 ± 2279.13	4199.09 ± 1883.36	462.21 ± 302.26	281.75 ± 131.10		
Group B (Porcilis PRRS IM)	4	7852.14 ± 2400.30	3703.49 ± 1424.05	449.81 ± 380.40	288.70 ± 217.99	ns	
	7	10,027.36 ± 3277.65	5439.56 ± 2147.07	579.48 ± 380.01	479.61 ± 297.45		
	10	8969.18 ± 2645.03	4597.08 ± 1993.06	518.82 ± 348.48	299.07 ± 130.23		
Group C (Diluvac ID)	4	7392.18 ± 2179.97	3788.80 ± 1642.64	427.59 ± 287.21	246.49 ± 107.33	ns	
	7	9464.64 ± 2893.23	4464.03 ± 1716.50	542.18 ± 458.52	347.98 ± 262.76		
	10	8870.62 ± 2615.96	4546.56 ± 1971.16	513.12 ± 344.65	295.79 ± 128.81		
Group D (Diluvac IM)	4	7856.80 ± 2541.17	4447.40 ± 1751.86	567.02 ± 421.01	410.84 ± 234.99	ns	
	7	9324.44 ± 2923.24	4961.77 ± 2298.55	545.90 ± 193.86	442.28 ± 246.95		
	10	10,006.70 ± 2769.45	6286.92 ± 2892.31	670.22 ± 385.91	302.86 ± 88.23		

ns: no significant differences.

3. Materials and Methods

3.1. Ethics

All animal care and use were reviewed and approved by the Ethical Committee of the Faculty of Veterinary Medicine, School of Health Sciences, University of Thessaly (approval number 98/19.12.2019).

3.2. Experimental Animals

The present study was carried out in a farrow-to-finish commercial pig farm (commercial hybrids of Large White x Landrace) suffering from positive unstable PRRSV status. One month before the beginning of the trial, the presence of PRRSV infection was confirmed by

collecting blood samples and using qRT-PCR (at ages of 4 weeks, 7 weeks, 10 weeks, and 13 weeks, 100% of the tested pigs were qRT-PCR-positive).

Totally, 104 suckling healthy piglets of 2 weeks of age were included in the trial, derived from 9 litters. The piglets were placed individually (within the litters) in the test groups equally based on the BW and sex, as well as the parity number of their sows (parities 1 to 5). The piglets were separated into four groups (Table 4) of 13 piglets, and two duplicates of the experimentation were performed (2 duplicates × 13 piglets × 4 groups/26 piglets per group). Diseased and significantly underweight piglets or piglets with abnormalities were not included in the trial.

Table 4. Experimental groups of the trial.

Group A (Porcilis PRRS ID	Group B (Porcilis PRRS IM)	Group C (Diluvac ID)	Group D (Diluvac IM)
0.2 mL Porcilis® PRRS (ID)	2 mL Porcilis® PRRS (IM)	0.2 mL of Diluvac Forte (ID)	2 mL of Diluvac Forte (IM)

The piglets were assigned individually (within the litters) to the groups, and they were ear-tagged as they came to hand. The piglets were allocated as they came to hand using a randomization list until the required number of piglets had been reached. All piglets were identified with two one-of-a-kind numbered ear tags. The color of the tags varied by production batch (week or batch of birth). Misplaced tags were replaced.

Piglets from the experimental and control groups were mixed and housed as usual. Piglets selected for blood sampling received differently colored ear tags. The identification of the study piglets was recorded. The piglets in the study were fed as standard on the farm, and water was available ad libitum.

The study was blinded because, after administration, the persons making the observations or estimations could not identify the vaccination group of the piglets. It was practically impossible to recognize individual piglets that were mixed in litters or pens except by their ear tag number.

3.3. Experimental Material

A commercial PRRSV MLV vaccine (Porcilis® PRRS, MSD Animal Health) was used in the current trial. This live attenuated lyophilized vaccine is approved for administration both via the IM or the ID injection in the neck area, as stated in the Summary of Product Characteristics (SPCs). Per vaccine, there was a dose of 2 mL (IM) or 0.2 mL (ID) of reconstituted lyophilized vaccine, the attenuated PRRSV strain DV was contained at titers $10^{4.0}$–$10^{6.3}$, and the tissue culture infective dose was 50%. Diluvac Forte® (MSD Animal Health, Rahway, NJ, USA) contains 75 mg/mL of dl-α tocopheryl acetate, and it is the adjuvant of the used vaccine. Diluvac Forte® was administered IM or ID to the piglets of the control groups.

3.4. Study Design

At 2 weeks of age, piglets were IM-vaccinated with 1 dose of Porcilis® PRRS, diluted in 2 mL of Diluvac Forte, or ID-vaccinated with the same vaccine, diluted in 0.2 mL of Diluvac Forte (Table 4). An IDAL (IntraDermal Application of Liquids, MSD Animal Health, Rahway, NJ, USA) device was utilized to perform the needle-free ID injection of experimental groups A and C. The pigs of the IM administration groups (B and D) were injected with an automatic syringe (standard fixed volume 2 mL), and a new, sterile needle (size approx. 0.9 × 13 mm) was used for each group.

3.5. Sampling/Laboratory Examinations

Blood samples were collected from each experimental group (three same ear-tagged piglets per group for two replicates) at 4, 7, and 10 weeks of age. All blood samples were

collected by jugular puncture using disposable syringes and needles. Serum was collected from all blood samples after centrifugation (10 min at 3000× *g*) and stored at −80 °C for further laboratory analysis.

Blood serum samples were subjected to nucleic acid extraction using the PureLink® Viral RNA/DNA Mini Kit (Invitrogen, Carlsbad, CA, USA). Extracts were examined for the PRRSV genome using a qRT-PCR assay [29]. Reactions were performed on a CFX96® Real-Time System (Bio-Rad Laboratories, Hercules, CA, USA) [29]. Cycle threshold (Ct) values were used as viral load estimates.

Serum samples were also tested by an ELISA for the quantitative determination of copeptin levels [Porcine CPP (Copeptin) ELISA Kit, Wuhan Fine Biotech Co., Ltd., Hubei, China], with 9.375 pg/mL sensitivity. This ELISA has high sensitivity and excellent specificity for detecting pig copeptin. No significant cross-reactivity or interference between pig copeptin and analogues was noticed. The detection range was 15.625–1000 pg/mL. Blood samples were handled and stored according to Good Laboratory Practices (GLPs).

Regarding the hematological variables, the packed cell volume (PCV) was assessed through the microhematocrit method, as previously described [30], and the numbers of white blood cells (WBCs) and platelets number (PLTs) were counted in blood smears stained with Giemsa [31]. In addition, a differential number of WBCs was calculated.

3.6. Statistical Analysis

Statistical analysis was performed with SPSS v.21. Data are expressed as mean ± standard deviation. Comparisons were performed using Kruskal–Wallis's test, while the Mann–Whitney U test was used for post hoc/multiple comparisons. Comparisons between more than two measurements of the same group over time were performed using Friedman's test and Wilcoxon's signed rank test as a post hoc test. All tests were two-sided. $p < 0.05$ was considered to indicate a statistically significant difference.

The distribution of continuous data was tested for normality using the Kolmogorov–Smirnov test, and they are shown as mean ± standard error and/or median and range (non-normal distribution). Comparisons for the copeptin levels were performed by applying the Kruskal–Wallis test, while the Mann–Whitney U test was utilized for post hoc/multiple comparisons. The comparison for the blood cell counts was performed using a one-way ANOVA, and for the post hoc comparisons, Tukey's HSD test was used. Significance was set at 0.05. Statistical analysis was performed in IBM (IBM Corp., Armonk, NY, USA).

Correlation between Ang II blood serum levels and PRRSV viral load, as measured by qRT-PCR, was evaluated by the non-parametric Spearman's correlation coefficient (ρ) using the commercial statistical software MedCal 9.2 software (MedCalc Software, Mariakerke, Belgium). Significance was set at 0.05. The strength of the relationship was ranked as follows: $\rho \leq 0.35$—weak correlations, 0.36 to 0.67—moderate correlations, and 0.68 to 1.0—strong correlations [32].

4. Discussion

Our study showed no discernible change in copeptin levels following either IM or ID vaccine administration. This lack of alteration may be attributed to adjuvants of the specific vaccine that have a minimal impact on the pituitary gland. As suggested in a previous study [33], the etiology of hypophysitis likely stems from vaccine adjuvants, once autoimmune and infiltrative granulomatous disorders are ruled out. In our previous study, the investigation of changes in piglet serum Ang II levels following ID and IM vaccination with the same tested commercial PRRSV MLV vaccine showed significant differences in Ang II in 7-week-old piglets [28]. The findings of this study provided evidence that the ID vaccination of piglets against PRRSV can induce diminished tissue damage, based on the lower levels of Ang II in the serum of ID-vaccinated piglets [28]. High Ang II levels cause nocturnal hypertension and can destroy the physiological spoon-shaped blood pressure via oxidative stress, AVP release, and sympathetic activation [34–36]. Copeptin is considered to be an indication of AVP levels since it is present in the bloodstream in

equimolar concentrations with AVP [37]. In contrast, in the present study, no significant differences in serum copeptin levels were noticed in the same group over time or among groups. Based on our results, the impact of ID or IM PRRSV vaccination of piglets with an MLV vaccine does not affect the levels of the released copeptin. Our findings indicate that the levels of the released copeptin are unaffected by the ID or IM PRRSV immunization of piglets receiving an MLV vaccination. Serum copeptin measurement, however, could not be suggested as a possible stress biomarker in pigs based on our findings. As an alternative non-invasive biomarker of anxiety in dogs, salivary copeptin levels have been suggested [18]. Additional research is required to examine the function of copeptin as a possible stress biomarker in pigs, as well as if there is any possible stress-associated dysregulation of HPA axis activity, mediated through AVP-dependent mechanisms. Our study has some limitations, including the sample size and the impossibility of carrying out the trial with PRRSV inoculation of the piglets under field conditions.

Regarding the blood cell counts, none were found to have a difference in the same group or among the groups. However, regarding the PCV values, Mayer et al. suggest that the AVP receptor 1B, which is expressed in hemopoietic stem and progenitor cells, may induce fast RBC production following specific events such as bleeding, drug toxicity, and chemotherapy [20]. Similar findings were made by Schill et al., who found that there is an association between copeptin and RBCs [19]. On the other hand, Winzeler et al. claimed in their research that chronic low AVP levels (measuring the copeptin concentration) associated with central diabetes insipidus and primary polydipsia do not affect the hemoglobin levels and the prevalence of anemia [21]. Furthermore, even though there was an increase in the number of WBCs and lymphocytes in groups A and B at the second time point of the blood sampling, this might be due to the vaccination and the interaction of the immune system with it [38]. However, copeptin seems to have a significant association with WBCs and the neutrophil count, as it has been described that AVP is involved in inflammation [19,22]. Moreover, copeptin concentration is associated with the number of PLTs, as AVP is involved in the activation of several coagulation factors and thrombocyte aggregation [19,23].

5. Conclusions

It seems that vaccination against PRRSV does not affect the levels of the released copeptin and the blood cell counts in ID- and IM-vaccinated piglets against PRRSV. However, further research is required, as the literature is limited to the effect of ID and IM PRRSV vaccination with MLV on the pituitary function and the released hormones.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/stresses4020023/s1, Figure S1: Comparison of ORF 5 sequences between the DV vaccine strain and the wild-type PRRSV strain of the trial farm: 90.7% sequence identity. Table S1: Results of qRT-PCR in blood samples at ages of 4, 7 and 10 weeks.

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Institutional Review Board Statement: All animal care and use are reviewed and approved by the Ethical Institutional Committee (Faculty of Veterinary Medicine, School of Health Sciences, University of Thessaly/approval number 98, date: 19 December 2019). The farm owner of the trial farm was informed in detail and provided written consent for using animals.

Informed Consent Statement: Written informed consent was obtained from the participants before starting the study.

Data Availability Statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Conflicts of Interest: Maragkakis G.G. was employed by MSD Animal Health from April 2018 to May 2019. Beyond that, the authors declare no conflicts of interest.

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