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Original Research

Vaccination-associated lameness in warmblood horses after intramuscular injection

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ABSTRACT

Background: Intramuscular vaccination is a routine component of equine medicine, but local muscle soreness may transiently affect gait symmetry. Objective data on vaccination-associated gait changes in horses are lacking.
Objectives: To investigate whether intramuscular vaccination induces measurable gait asymmetries depending on injection site, to inform recommendations on vaccination site selection and short-term exercise management.
Methods: In this prospective, randomised, blinded, placebo-controlled study, eighteen clinically sound Warmblood horses were enrolled and received an intramuscular vaccination or a 2.0mL saline injection into either the musculus pectoralis descendens or musculus semitendinosus. Objective gait analysis using body-mounted inertial measurement units was performed at baseline and 8, 24, 48, 72 and 96 hours after injection. Vertical displacement asymmetries of the head, withers and pelvis were analysed using predefined clinical relevance thresholds.
Results: Fourteen horses were included in the final analysis (pectoralis: $n_{experimental}$=8/$n_{control}$=5; semitendinosus: $n_{experimental}$=6/$n_{control}$=3). Vaccination into the musculus semitendinosus resulted in a transient increase in hindlimb push-off asymmetry. Mean pelvic push-off asymmetry increased from 5.47 mm at baseline to 10.57 mm at 48 hours post-vaccination ($P < 0.001$) and returned to baseline by 96 hours. No clinically relevant changes in gait symmetry were detected following vaccination into the musculus pectoralis descendens or after saline injection at either site, despite an isolated statistically significant change in the semitendinosus control group at timepoint 96.
Conclusion: Vaccination into the musculus semitendinosus resulted in a transient increase in hindlimb push-off asymmetry after 48 hours. These findings support a short reduction in training for at least 72 hours following vaccination.

1. Introduction

Vaccination is an effective prophylactic strategy in equine health management [1,2]. Although generally safe, local reactions such as muscle soreness may occur [1–5]. The Fédération Equestre Internationale (FEI) recommends a waiting period of at least seven days before competition following influenza vaccination to minimise the risk of virus shedding after administration of a live vaccine [6]. Beyond this, evidence-based recommendations regarding post-vaccination management in horses are limited. In clinical practice, veterinarians and vaccine manufacturers often advise reducing exercise for 24–48 hours post-vaccination, as mild local reactions are typically reported within the first 24 hours and resolve within 48 hours [7,8]. These recommendations are based on clinical experience and may vary slightly between sources.

During routine gait analyses performed by members of our research group, several Shetland ponies exhibited mild to moderate hindlimb push-off lameness five days post-vaccination into the musculus semitendinosus, suggesting that this phenomenon warrants further investigation under controlled conditions [9].

In human medicine, transient muscle soreness following intramuscular vaccination is well documented, and temporary exercise restriction is commonly recommended, although evidence-based guidance on optimal duration is limited [10–13].

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In horses, discomfort or pain can lead to changes in the movement pattern and lameness. Objective gait analysis quantifies such changes by calculating the difference in vertical displacement between left and right steps—measured as HDmin/HDmax (head), WDmin/WDmax (withers), and PDmin/PDmax (pelvis) [14,15]. In hind limb lameness, a differentiation is made between impact (PDmin) and push-off (PDmax) lameness related to the asymmetric movement during different parts of the gait cycle [16].

In horses, the musculus (M.) pectoralis descendens, M. semitendinosus, M. serratus ventralis cervicis and M. gluteus medius are commonly used for intramuscular injections [4,17,18]. Due to poor gravitational drainage along fascial planes, local adverse reactions in the gluteal or cervical muscles are difficult to treat, and these sites should therefore generally be avoided for vaccination [3,18]. Consequently, the M. pectoralis descendens and M. semitendinosus are commonly recommended injection sites, as local complications are more accessible and easier to manage [5,19–21]. Functionally, the M. semitendinosus acts as an extensor of the hip, stifle and tarsal joints in the supporting limb, thereby propelling the trunk forward. In the swinging limb, it functions as a flexor of the stifle joint and guides the limb both forward and backward. The M. pectoralis descendens acts as an adductor, as well as a protractor of the limb [22].

The aim of this prospective, double-blinded, placebo-controlled study was to investigate whether routine vaccination induces measurable gait asymmetries in Warmblood horses.

We hypothesised that vaccination would result in muscle-specific gait asymmetries, reflected as changes in vertical head movement (HDmin/HDmax) following injection into the M. pectoralis descendens and changes in vertical pelvic movement (PDmin/PDmax) following injection into the M. semitendinosus. We further hypothesised that any observed asymmetries would return to baseline within 96 hours, consistent with reported timelines of post-vaccination muscle soreness in equine and human medicine [7,8,23,24].

2. Methods and materials

2.1. Ethical approval

The study protocol was approved by the Animal Welfare Body Utrecht (Instantie voor Dierenwelzijn Utrecht) under the Dutch Central Committee for Animal Experiments (CCD) (license number: AVD10800202013735; corresponding work protocol number: 13735-1-04; Approval date: 6 April 2022).

2.2. Horses

A total of 18 Warmblood horses were included in the study. None of the horses had a recent history of lameness and had not been vaccinated recently. The horses underwent comprehensive clinical and orthopaedic examinations by an experienced orthopaedic (DVM, Dipl. ECVS) one day before the start of the study to confirm their health status and verify their soundness. The orthopaedic pre-examination of the horses consisted of a visual inspection for lameness at the walk and trot on a straight line on hard and soft ground.

Exclusion criteria included any signs of current lameness, a history of lameness within the last months, or recent vaccination against influenza or tetanus (less than six months prior to the study). Horses showing clinical signs of systemic disease were also excluded.

2.3. Study design

This study was conducted as a prospective, randomised, blinded, placebo-controlled trial.

All 18 horses participated in the vaccination group (VAX). After a four-week interval, a subset of nine of these horses additionally participated in a placebo control group (CONTROL), resulting in a two-phase study design using the same horses.

Respecting the principle of reduction in animal experimentation (3Rs), this design allowed reuse of horses for the control phase while minimising the number of animals and avoiding overlapping effects between study phases.

2.4. Vaccination and injection sites

During the vaccination study phase (VAX), allocation to injection site (M. pectoralis descendens or M. semitendinosus) was performed using block randomisation implemented in a custom-written MATLAB script to ensure balanced group sizes. The side of injection (left or right) was allocated using simple randomisation.

In the vaccination group, horses received an intramuscular injection into the M. pectoralis descendens ($n_{experimental} = 9$) or the M. semitendinosus ($n_{experimental} = 9$).

Vaccinations were administered according to the horses' regular vaccination schedule using an influenza vaccine (Equilis Prequenza) or an influenza-tetanus vaccine (Equilis Prequenza Te).

In the placebo control phase (CONTROL), horses received a saline injection into the contralateral muscle relative to the vaccination injection site. Consequently, the control group consisted of five horses receiving the injection into the M. pectoralis descendens ($n_{control} = 5$) and four horses receiving the injection into the M. semitendinosus ($n_{control} = 4$).

All injections were administered using a 20 G x 40 mm needle and a 2 mL syringe. Injections were performed by an independent veterinarian who was not otherwise involved in the study.

2.5. Gait analysis protocol

Gait measurements were performed at baseline (T00) and at 8 h (T08), 24 h (T24), 48 h (T48), 72 h (T72), and 96 h (T96) after injection (see Fig. 1 [4,17,18]).

Horses were trotted in hand on both hard and soft straight-line surfaces under consistent environmental conditions. All measurements were conducted in a covered trotting arena.

After the baseline measurement, each horse received either a vaccination or saline injection at its assigned injection site.

Gait data were collected using body-mounted inertial measurement units (IMUs) sampling at 200 Hz and EquiMoves® software. The system included seven nodes (ProMove mini, Inertia Technology, Oldenzaal, The Netherlands) placed at the following locations: head, withers, sacrum, and the lateral aspect all four cannon bones. The head sensor was mounted centrally on the neckpiece of the bridle, the withers sensor on the dorsal midline of the girth, and the sacrum sensor directly on the skin using an animal pollster between the tubera sacrale. Limb sensors were secured laterally on the metacarpal and metatarsal regions using elastic spats.

Throughout the study, horses were housed in box stalls and turned out daily for 90 minutes in individual 3 × 3 m paddocks.

2.6. Clinical assessment

Alongside the objective measurements, all horses were assessed for visible lameness by an experienced orthopaedic clinician (DVM, Dipl. ECVS), and daily clinical exams were conducted each morning prior to data collection.

Objective gait analysis, as well as the orthopaedic and clinical examinations, were performed by the same blinded operators and under the same conditions for the duration of the study.

2.7. Outcome measures

To quantify gait asymmetry, vertical movement of the head, withers and pelvis was recorded using IMUs and further analysed using custom

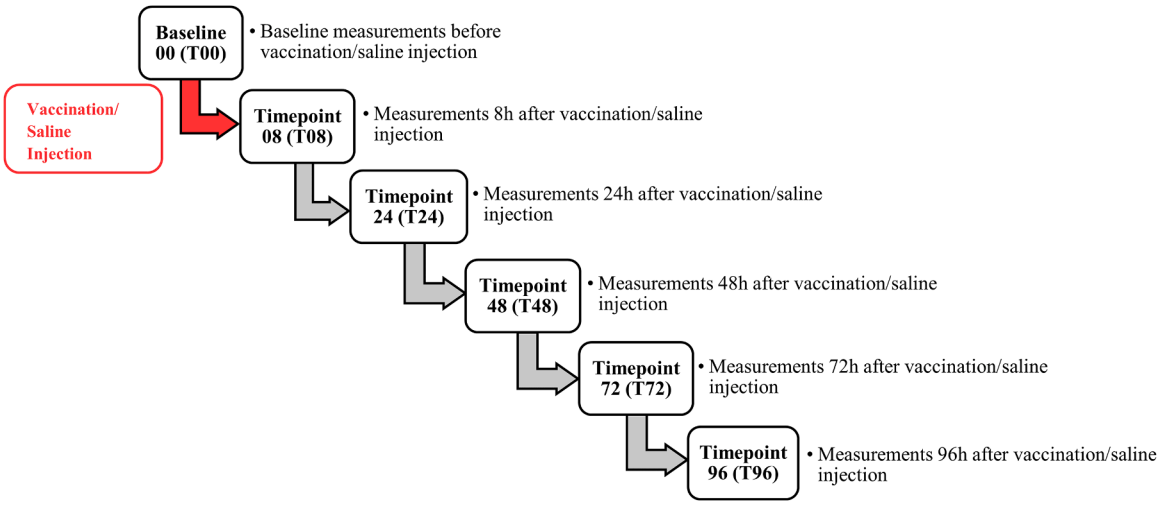

Fig. 1. Timeline of gait measurements: Horses were evaluated while trotting using body-mounted inertial measurement units at baseline and at predefined timepoints following vaccination or saline injection. Baseline measurements (T00) were obtained prior to administration. Vaccination or saline injection was then administered (red marker). Subsequent measurements were performed at 8 h (T08), 24 h (T24), 48 h (T48), 72 h (T72), and 96 h (T96) post-vaccination.

written scripts. The thresholds used in this study to classify movement asymmetry as clinically relevant were HDmin/HDmax $\geq |15|$ mm and WDmin/WDmax/PDmin/PDmax $\geq |6|$ mm, with trial mean larger than the standard deviation). A change of $\geq |5|$ mm from baseline at any timepoint was considered a relevant clinical change. The suitability of these conservative thresholds is also supported by previous studies [25–28].

2.8. Data analysis and statistics

Raw IMU data were processed using MATLAB and all statistical analyses were performed in RStudio. Upper-body asymmetry metrics and temporal stride parameters were extracted from each stride-segmented signal. For the upper body asymmetry parameters, the stride split vertical displacement trajectories of the head, withers, and sacrum were used. For all three locations, the difference between the vertical displacement minima (HDmin/WDmin/PDmin) and vertical displacement maxima (HDmax/WDmax/PDmax) were calculated (see Table 1 and Fig. 2).

Pilot data from Shetland ponies were used to perform an a priori power analysis using the 'pwr' package in R, indicating that a sample size of seven horses per vaccination group would be required to detect clinically relevant changes in asymmetry parameters [9].

The data from the left side injection sites were mirrored to match the data from the right-side injection sites. This was necessary because left-sided lameness results in negative vertical displacement asymmetry values, while right-sided lameness results in positive vertical displacement asymmetry values. By mirroring the left-sided data, we standardized it to show positive displacement, making it easier to compare across all horses.

Linear mixed models (random effect = horse, fixed effect = timepoint, α=0.05) were used to analyse the differences in upper body asymmetry between baseline and the different timepoints, separately for the pectoralis and semitendinosus muscles. Model fit was evaluated using AIC values, Q-Q plots and residual plotting. The false discovery rate was utilized for adjusting multiple comparisons.

Study phase (VAX and CONTROL) was not included as a fixed effect, as sessions were separated by a four-week interval and no carry-over effects were expected.

Table 1
Definitions of HDmin, PDmin, HDmax and PDmax and the units they are measured in.

VARIABLE	UNITS	DESCRIPTION
HDmin/WDmin/ PDmin = MinDiff head/ withers/sacrum	millimeters (mm)	The difference between the minimum vertical positions reached by the head/ withers/sacrum during the left versus right stride half-cycle
HDmax/WDmax/ PDmax = MaxDiff head/ withers/sacrum	millimeters (mm)	The difference between the maximal vertical positions reached by the head/ withers/sacrum during the left versus right stride half-cycle
Negative variable	millimeters (mm)	Left-sided asymmetry
Positive variable	millimeters (mm)	Right-sided asymmetry

3. Results

Initially 18 horses were enrolled in this study. During the vaccination phase (VAX), three horses completed the baseline examination and received their assigned vaccination, but left the university before timepoint 08 (T08) for reasons unrelated to the study. Therefore, they did not complete the follow-up measurements. One vaccinated horse developed lameness during the study period and a subsequent clinical examination confirmed an orthopaedic cause unrelated to the vaccination. This horse was therefore excluded from the analysis. Consequently, data from fourteen horses were included in the final analysis of the vaccination group (VAX), with eight horses in the pectoralis group ($n_{experimental}$=8) and six horses in the semitendinosus group ($n_{experimental}$=6).

Nine horses additionally participated in a second study phase in the placebo control group with a four-week interval (CONTROL). One of these horses was excluded during baseline assessment due to unrelated lameness. Thus, eight horses were included in the final analysis of the control group (CONTROL), with five horses in the pectoralis group ($n_{control}$=5) and three horses in the semitendinosus group ($n_{control}$=3). Final group allocation is summarised in Supplementary Table S2.

Due to the limited space in the arena, a reduced number of consecutive strides was recorded for each of those horses on the soft surface. As a result, our statistical analyses were exclusively conducted on data obtained from the hard surface. Soft surface analyses of the primary outcome parameters (PDmax and HDmin) are provided in the Supplementary Materials (see Fig. S7 and Fig. S8).

All the median data and p-values from the measurements on the hard surface can be found in the Supplementary Materials (Table S3 and S4). In addition, supplementary figures illustrating hard surface data for PDmin and HDmax are provided (see Supplementary Materials Fig. S5

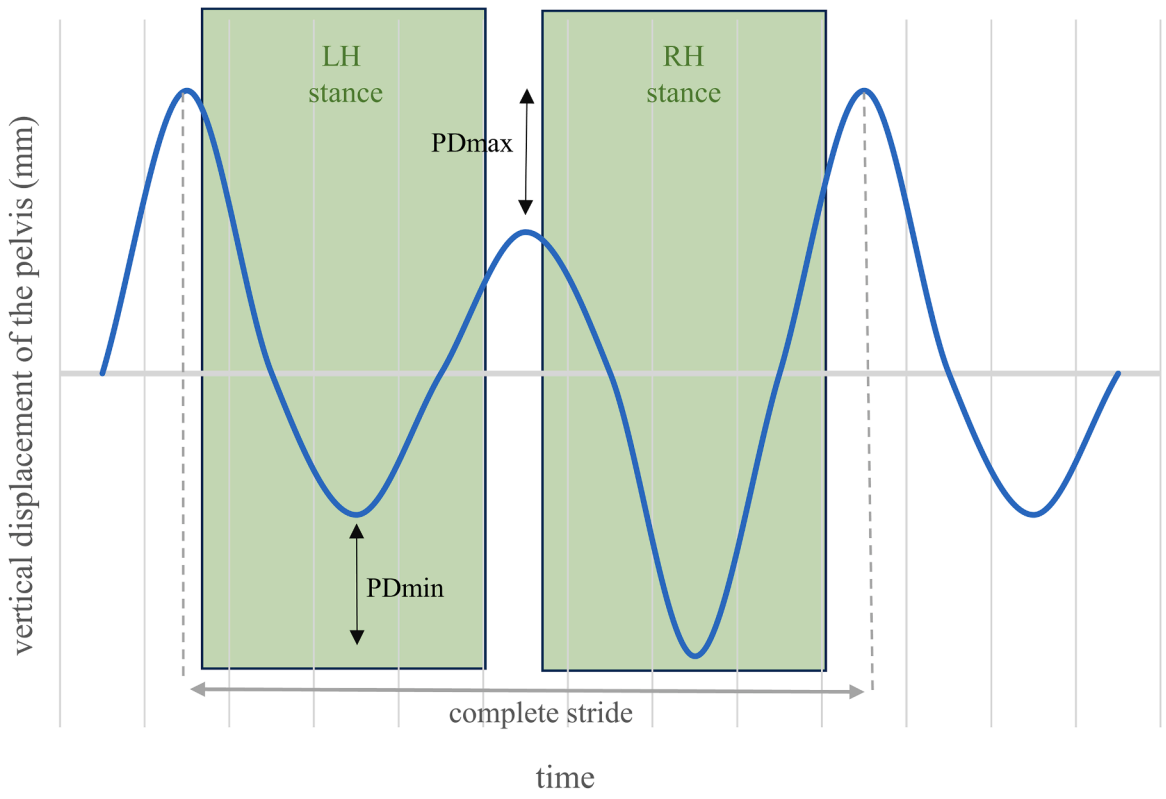

Fig. 2. Example of the vertical displacement of the sacrum sensor at one complete stride of a trotting horse. The graph represents a push-off and impact asymmetry of the right hind.
LH, left hind; RH, right hind;.

and Fig. S6).

Although several parameters showed statistically significant differences ($P < 0.05$) over time, these changes did not meet the predefined thresholds for clinically relevant asymmetry (HDmin/HDmax $\geq$ 15 mm; WDmin/WDmax/PDmin/PDmax $\geq$ 6 mm; change from baseline $\geq$ 5 mm). Therefore, statistical significance alone was not considered indicative of a clinically meaningful asymmetry change.

3.1. *Musculus pectoralis descendens*

No clinically relevant gait asymmetries were detected following vaccination into the M. pectoralis descendens. All measured parameters remained within the predefined thresholds, and no horse exhibited observable lameness at any timepoint (see Fig. 3 and Table S3 and S4 and Fig. S5 in the Supplementary Materials)).

3.2. *Musculus semitendinosus*

Vaccination into the M. semitendinosus led to a significant and transient increase in hindlimb push-off asymmetry (PDmax), with a peak at 48 hours. PDmax increased from 5.47 mm at baseline (T00) to 10.57 mm at T48 ($P < 0.001$), representing a +5.10 mm change that exceeded the predefined clinical relevance threshold. Values had returned to baseline levels by 96 hours post-vaccination (see Fig. 4 and Tables S3 and S4 in the Supplementary Materials).

No clinically relevant changes were detected for the other asymmetry parameters at any timepoint, suggesting that the observed asymmetry was specific to the hindlimbs and time dependent (see Fig. S6 in the Supplementary Materials).

3.3. *Control group*

No clinically relevant gait asymmetry was observed in the M. pectoralis descendens control group (see Fig. 3 and Fig. S5 in the Supplementary Materials).

In contrast, in the M. semitendinosus control group, PDmax values increased from 2.48 mm at baseline (T00) to 8.00 mm at 96 hours (T96), representing a +5.52 mm change ($P < 0.001$, see Fig. 4 and Tables S3 and S4 in the Supplementary Materials).

4. Discussion

This study is the first to demonstrate a potential relationship between vaccination-associated gait asymmetries and injection site in horses. The results show that intramuscular vaccination into the M. semitendinosus led to a transient, significant push-off-type hindlimb asymmetry (PDmax), peaking at 48 hours ($P < 0.001$) and resolving by 96 hours, whereas vaccination into the M. pectoralis descendens did not result in clinically relevant forelimb gait asymmetry as assessed by vertical head movement. The initial hypothesis was therefore only partially supported. Notably, the increase in PDmax in the semitendinosus vaccination group exceeded the predefined threshold for clinical relevance, indicating that the detected asymmetry was not just a statistical finding, but reflected a clinically meaningful change.

Despite the fact that the observed push-off lameness in this study was reversible, it nevertheless indicates transient discomfort and a mild impairment of movement. These findings suggest that, following intramuscular vaccination into the M. semitendinosus a short period of reduced training intensity for 72 hours may be advisable to support both performance and welfare of horses.

Although clinically relevant asymmetry was detected only after semitendinosus vaccination in the present study, it is important to recognise that the absence of measurable upper body asymmetry does not exclude the presence of local discomfort. Particularly in the pectoral muscle, post-vaccination soreness may still occur without producing detectable changes in vertical displacement. This distinction is clinically

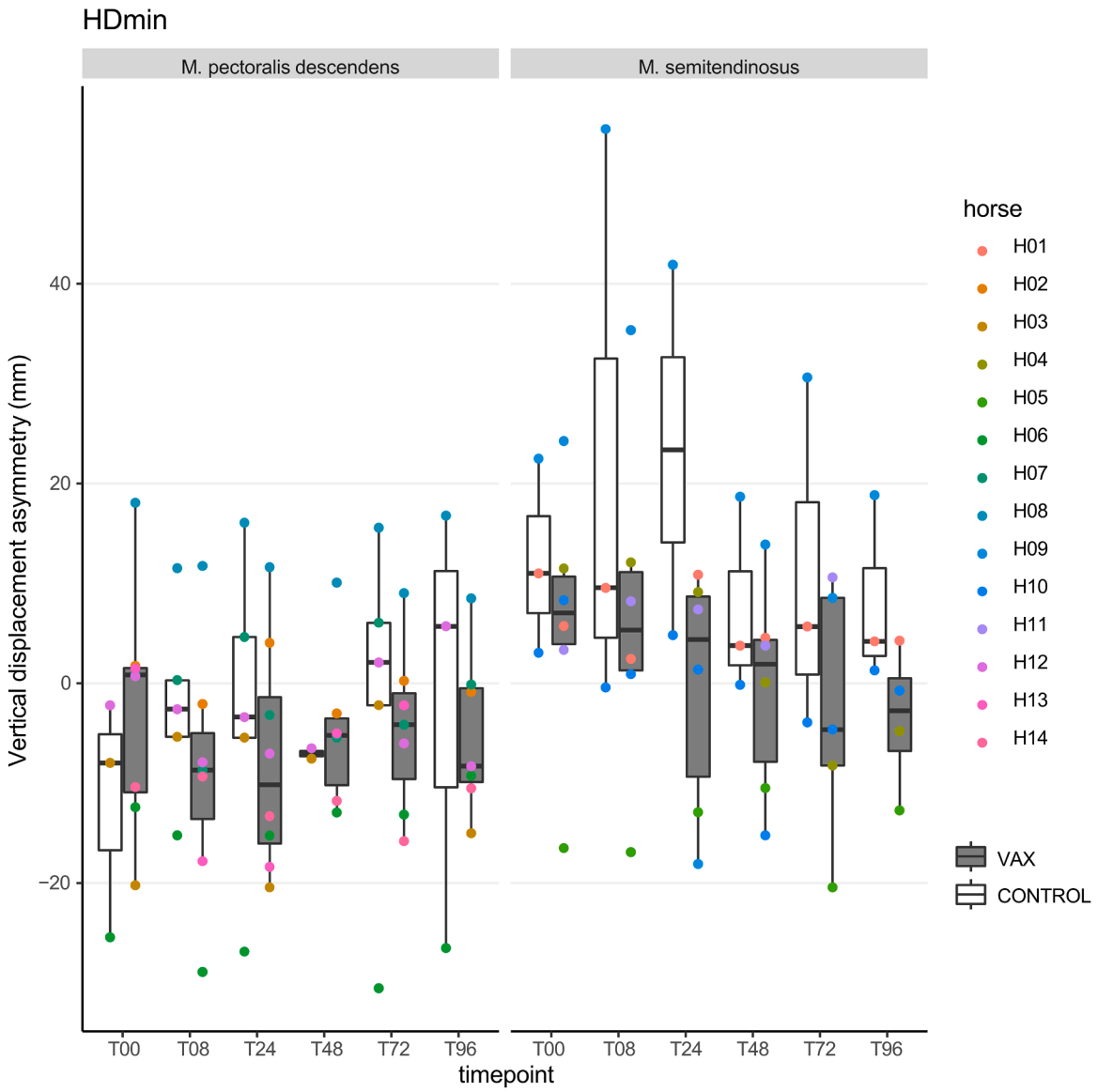

Fig. 3. Vertical head displacement asymmetry (HDmin, mm) measured at baseline (T00) and at different timepoints after intramuscular injection into the M. pectoralis descendens (left panel) and M. semitendinosus (right panel). Vaccinated horses (VAX, grey boxes) and control horses (CONTROL, white boxes) are shown separately. Boxes represent median and interquartile range; whiskers indicate range; coloured dots represent individual horses.
Vaccination group: M. pectoralis n =8, M. semitendinosus n = 6; Control group: M. pectoralis n = 5, M. semitendinosus n = 3. Due to occasional missing trials in the originally extracted dataset, the number of data points may vary between timepoints. - T00, baseline; T08, 8 hours after vaccination/saline injection; T24, 24 hours after vaccination/saline injection; T48, 48 hours after vaccination/saline injection; T72, 72 hours after vaccination/saline injection; T96, 96 hours after vaccination/saline injection; H01-H14, horse-ID; M., Musculus; mm, millimetres;

relevant, as soreness that does not cross the threshold for measurable gait asymmetry may still affect comfort, handling, or performance, even though it does not manifest as measurable gait asymmetry. Previous pharmacovigilance reports support this interpretation, describing local injection-site reactions such as local pain, which are the most frequently reported adverse events and are occasionally accompanied by stiffness or transient gait irregularities, especially after vaccination into the M. pectoralis descendens [29–32]. Reported cases predominantly involve equine influenza vaccines, alone or in combination with tetanus, which corresponds to the vaccine types used in the present study. In the present study, however, no comparable alterations were detected after pectoral vaccination, suggesting that potential local reactions in this region were either mild and insufficient to cause measurable vertical gait asymmetries or caused other changes in the gait pattern not investigated within the current study.

The functional roles of the two muscles likely explain the different effects observed post-vaccination at the two injection sites. The M. semitendinosus is a primary extensor of the hip, stifle, and hock joints during weight-bearing, and therefore plays a critical role in propulsive motion [21]. Any local discomfort in this muscle is likely to affect hindlimb push-off, making changes in pelvic movement detectable. By contrast, the M. pectoralis descendens primarily acts as an adductor and stabiliser of the forelimb, connecting it to the trunk as part of the thoracic sling [21]. Consequently, local reactions in this muscle are less likely to result in measurable upper body asymmetries during trotting.

Findings from the control groups further support the time- and muscle-specific interpretation of the results. No clinically relevant asymmetry was observed in the M. pectoralis descendens control group. In the M. semitendinosus control group, an isolated increase in PDmax occurred at 96 hours, driven by a single horse and occurring outside the expected window for injection-related reactions. Given the small group size and delayed onset, this finding is unlikely to represent a

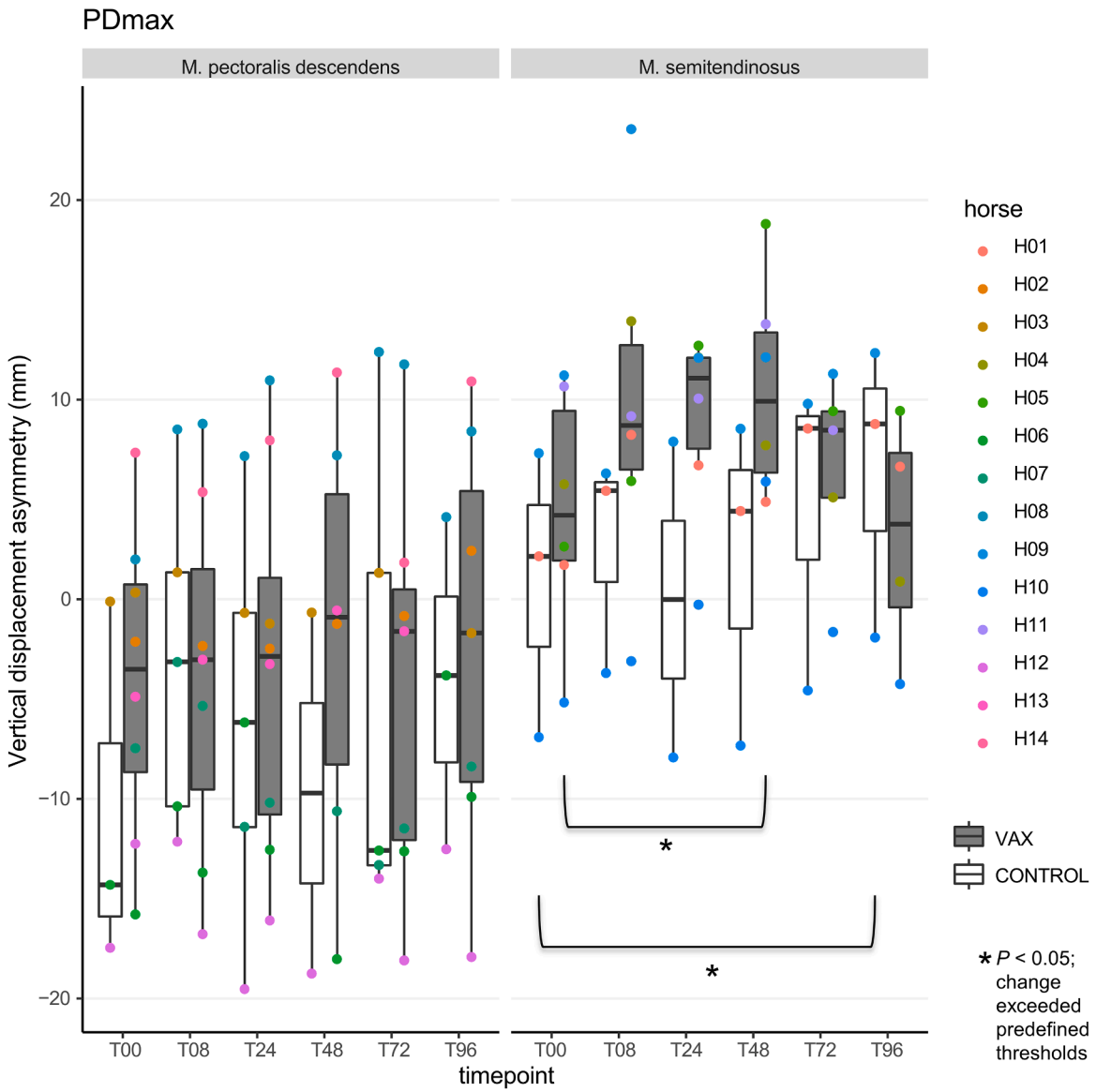

Fig. 4. Vertical pelvic displacement asymmetry (PDmax, mm) measured at baseline (T00) and at different timepoints after intramuscular injection into the M. pectoralis descendens (left panel) and M. semitendinosus (right panel). Vaccinated horses (VAX, grey boxes) and control horses (CONTROL, white boxes) are shown separately. Boxes represent median and interquartile range; whiskers indicate range; coloured dots represent individual horses.
*, indicates $P < 0.05$ compared with baseline and exceeding the predefined asymmetry thresholds. Vaccination group: M. pectoralis n =8, M. semitendinosus n = 6; Control group: M. pectoralis n = 5, M. semitendinosus n = 3. Due to occasional missing trials in the originally extracted dataset, the number of data points may vary between timepoints. - T00, baseline; T08, 8 hours after vaccination/saline injection; T24, 24 hours after vaccination/saline injection; T48, 48 hours after vaccination/saline injection; T72, 72 hours after vaccination/saline injection; T96, 96 hours after vaccination/saline injection; H01-H14, horse-ID; M., Musculus; mm, millimetres;

physiological effect of saline injection. This is supported by previous studies in horses and humans as well as clinical recommendations, which show that local post-injection reactions typically occur within the first 24–72 hours and resolve shortly thereafter, making a delayed response at 96 hours more likely to reflect an unrelated finding [7,8,23,24,33].

Taken together, these results suggest that the observed gait asymmetries were not attributable to the injection procedure alone, but rather to the immune response induced by vaccination. Both vaccine formulations used in this study share the same adjuvant system and differ only by the inclusion of tetanus toxoid in the combination vaccine [34,35]. According to manufacturer information, both products exhibit comparable adverse reaction profiles, involving transient local reactions such as injection site pain [34,35]. As the study was not powered to detect vaccine-specific effects, all vaccinated horses were analysed together, and formulation-related differences cannot be excluded.

Previous studies in human and veterinary medicine indicate that transient local adverse reaction such as pain, redness, nodules, swelling and abscess formation at the injection site reflect vaccine-induced immune activation, which in adjuvanted vaccines may be associated with local inflammatory responses [33,36,37].

At the same time, the mechanical component of the injection process should be considered. While the observed effects are most likely related to vaccine-induced immune responses, mechanical aspects of intramuscular injection may also contribute to local discomfort and should be considered when interpreting post-vaccination reactions [38].

Additionally, it's crucial to highlight that while objective gait analysis provides valuable supplementary information for clinicians in their decision-making process, it does not and cannot supplant the expertise of a veterinarian. The veterinarian holds the responsibility for the final

assessment and determination of whether the identified asymmetry should be classified as 'lameness' or not [15,39,40].

This study highlights the potential clinical relevance of transient post-vaccination lameness and supports recommendations for post-vaccination rest periods.

4.1. Main limitations of study

This study has several important limitations that should be considered when interpreting the results. Although an a priori power calculation indicated that seven horses per vaccination group were required, the final sample size used for data analyses was smaller due to exclusions and horses leaving the university after the vaccinated. Consequently, group sizes, particularly in the semitendinosus and control groups, were smaller than anticipated, and the findings should therefore be interpreted with caution. The semitendinosus vaccination group almost reached the intended sample size, and the detection of a significant change despite the reduced number suggests that the observed effect was consistent within this group. In contrast, the smaller control group sizes, particularly for the semitendinosus muscle, as this group was the smallest, should be considered when interpreting comparisons between vaccinated and control horses.

Although two different vaccines (influenza (Equilis Prequenza) or influenza/tetanus combination vaccine (Equilis Prequenza Te)) were used, the study was not designed or powered to compare vaccine-specific effects. Therefore, all vaccinations were analysed together, and potential differences between vaccine formulations cannot be excluded.

Other local injection site reactions such as swelling, pain on palpation or local hyperthermia were not systematically assessed. Therefore, a direct association between objective gait asymmetries and local inflammatory reactions could not be evaluated.

Furthermore, the investigation was also restricted to two injection sites, and reactions at other commonly used sites may differ.

Finally, the study population consisted predominantly of mares (93%), which limits conclusions about possible sex-related effects. However, the single gelding included in the study showed responses comparable to those of the mares, suggesting that major sex differences are unlikely.

5. Conclusion

Within the limitations of this study, routine intramuscular vaccination into the M. semitendinosus resulted in a transient, measurable push-off–type hindlimb asymmetry. This effect occurred 48 hours post-vaccination and resolved by 96 hours.

In contrast, no clinically relevant upper body asymmetry was observed post-vaccination into the M. pectoralis descendens. Likewise, no relevant asymmetries appeared in either control group. These findings suggest that the observed lameness was vaccine-related rather than injection-related.

Although clinically relevant gait asymmetry was only detected following M. semitendinosus injection, transient local muscle discomfort may also occur post-vaccination into other muscle groups without manifesting as measurable upper body asymmetry. From a clinical and welfare perspective a short period of reduced exercise for approximately 72 hours post-vaccination therefore appears advisable, regardless of the injection site, and particularly when the M. semitendinosus is used as injection site.

As adverse reactions like muscle soreness or pain have been reported for various equine vaccines, similar transient effects may occur with other formulations. Future studies with larger sample sizes, additional vaccine types, and more injection sites are warranted to confirm and refine these findings.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the authors used an AI-assisted tool in order to support language refinement and text formulation. After using this service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Ethics in publishing statement

The authors confirm that this manuscript complies with Elsevier's ethical guidelines for journal publication. The study was conducted in accordance with relevant ethical standards and approved by the appropriate institutional animal ethics committee. All procedures involving animals were performed in accordance with applicable laws, guidelines and regulations.

CRediT authorship contribution statement

J. Lenarz: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Data curation. **I.H. Smit:** Writing – review & editing, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M. Rhodin:** Writing – review & editing. **C. Lischer:** Writing – review & editing. **M.C. Fugazzola:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jevs.2026.105820.

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