A Double-Blinded Positive Control Study Comparing the Relative Efficacy of 2.5% Polyacrylamide Hydrogel (PAAG) Against Triamcinolone Acetonide (TA) And Sodium Hyaluronate (HA) in the Management of Middle Carpal Joint Lameness in Racing Thoroughbreds

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Journal of Equine Veterinary Science 107 (2021) 103780

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

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ARTICLE INFO

Article history:
Received 25 February 2021
Received in revised form 17 September 2021
Accepted 20 September 2021
Available online 24 September 2021

Keywords:
Osteoarthritis
Horse
Polyacrylamide
Joint
Synovitis
Symptom-Modifying Osteoarthritis Drug
Carpus
Lameness

ABSTRACT

The purpose of this prospective double-blinded positive control study was to compare the efficacy of 2.5% polyacrylamide hydrogel (2.5% PAAG) in the management of middle carpal joint lameness in Thoroughbreds against treatments of triamcinolone acetonide (TA) or sodium hyaluronate (HA). A total of 31 flat-racing Thoroughbreds with lameness (grade 1-3/5) localized to the carpus by intra-articular analgesia were selected. Following a radiological assessment of the carpi confirming the absence of fragment/fracture, the horses were randomly assigned for intra-articular treatment with either 2 ml of 2.5% PAAG, 12 mg TA or 20 mg HA (followed by two further intravenous treatments of 40 mg, at weekly intervals in the HA group only), by a treating veterinarian. All horses were rested for 48 hours post-treatment and then re-entered an unaltered training regimen. Subsequent examinations at 2, 4, and 6 weeks were performed by a blinded examining veterinarian for all groups, while horses treated with 2.5% PAAG were monitored for 12 weeks for recurrence of lameness. Significantly more joints treated with 2.5% PAAG were lame free (83%) at 6 weeks compared to TA (27%; $P = .007$) and to HA (40%; $P = .04$). There was no significant difference between TA and HA groups at any time. All the joints treated within 2.5% PAAG that were lame free at 6 weeks (10/12) were still lame-free at 12 weeks. In conclusion, treatment with 2.5% PAAG led to statistically superior results compared to TA and HA in the management of selected middle carpal joint lameness in flat-racing Thoroughbreds, with therapeutic effects persisting up to 12 weeks.

1. Introduction

2.5% polyacrylamide hydrogel (2.5% PAAG) is a non-toxic, non-particulate, homogenous polymer, consisting of 97.5% sterile water and 2.5% cross-linked polyacrylamide hydrogel. In small quantities, 2.5% PAAG is biocompatible when used for soft tissue augmentation in human medicine [1,2]. Animal studies using histological examination reveal its integration into the synovium layers in both osteoarthritic and non-osteoarthritic equine synovial joints through

Conflict of Interest Statement: Leigh Travis de Clifford – see attached document. Jason Nicholas Lowe – see attached document. Innovative Medical Solutions part-funded the research and owns the distribution rights to Arthramid® Vet in Australasia.

Animal Ethics Approval: Research was approved by the Animal Care and Ethics Committee of the Secretary NSW Department of Industry (TRIM 17/546). This study was performed under VICH GL9 Good Clinical Practice (GCP) standards, being verified and monitored by an independent Contract Research Organization (CRO). The study was approved by the Animal Research Authority and AEC from NSW license number TRIM 14/1284.

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https://doi.org/10.1016/j.jevs.2021.103780

a combination of vessel in-growth and molecular water exchange, with resultant deposition of collagen fibres [3,4].

Few studies have shown a corresponding alleviation of clinical signs of lameness using 2.5% PAAG treatment in naturally occurring disease. Distal interphalangeal, metacarpophalangeal, metatarsophalangeal, carpal and tarsocrural joints have been treated, with the efficacy of a single intra-articular dose of 2.5% PAAG lasting up to 24 months post-administration [5–8]. Although these studies were conducted on sport horses, racehorses have also been successfully treated using 2.5% PAAG. Recently, out of 49 flat-racing Thoroughbreds treated with 2.5% PAAG in the middle carpal, radiocarpal and metacarpophalangeal joints, 65.3% were lame-free at 24 weeks' post-treatment [9].

Despite this, these studies have several limitations, including not being randomized, blinded, not being conducted in a controlled setting or, not using controls. Furthermore, individuals in some studies were rested from ridden exercise for periods of up to one month, which could in itself have resulted in resolution of lameness in some cases. This prospective double-blinded positive control study aimed to investigate the efficacy of 2.5% PAAG compared with triamcinolone acetonide (TA) and sodium hyaluronate (HA) in the management of naturally occurring middle carpal joint lameness in flat-racing Thoroughbreds. The hypothesis was that there would be a significant difference between treatments during the study period.

2. Materials and Methods

2.1. Case Selection

All horses enrolled were flat-racing Thoroughbreds ≥24 months of age, trained at a single commercial facility, and that had been in training for at least 12 weeks before admission, being at a stage where they were galloping regularly. Enrolment examinations were scheduled once weekly, with all horses deemed to be underperforming during galloping exercise presented to the examining veterinarian for an assessment 48 hours later. Horses were assessed for lameness, joint effusion and reaction to carpal flexion and were selected if the source of lameness was isolated to the middle carpal joint. This included performing intra-articular analgesia using 100 mg of mepivacaine hydrochloride (Mepivicaine)[1] via a dorsolateral approach with re-examination after 5 to 10 minutes. Unilateral or bilateral middle carpal joint lameness were included. Throughout the entire study, all lameness assessments were performed on the same firm bitumen surface, with the horses evaluated at the trot in a straight line. Horses with bilateral lameness were included as long as the lameness was isolated to both middle carpal joints using intra-articular analgesia in all cases.

All horses selected for the study had a standard radiographic series of both carpi taken, including dorso-palmar, dorsolateral-palmaromedial, dorsomedial-palmarolateral, flexed latero-medial, dorso-30°-proximal-dorsodistal and dorso-70°-proximal-dorsodistal projections. The radiographs were assessed, and horses enrolled in the study if the absence of osteochondral fragments or fractures was confirmed.

Horses were excluded from the study if osteoarthritis was secondary to a joint infection, if the horse had surgery of the affected joint within 12 weeks of the enrolment date, or if the horse had intra-articular medication into any joint within 4 weeks of the enrolment date. Throughout the study period, horses were not administered any form of medication that could interfere with the assessment of lameness. All horses enrolled in the study were rested for 48 hours post-treatment, before continuing with their training regime commiserate with their response to treatment. A normal training regimen included galloping exercise every third day, with light canter and trot work on intervening days and swimming exercise at least once a week. Horses rested on Sundays of each week, which was consistent with standard training practices. The trainer was blinded to treatment with agent's/owner's giving informed written consent for enrolment in the study.

2.2. Variables Assessed

Three different parameters were assessed: lameness, joint effusion and reaction to carpal flexion. Lameness was considered the principal clinical parameter and resolution of lameness would be the main treatment objective. Lameness was graded as per the American Association of Equine Practitioner's guidelines, on a scale of 0 to 5 [10]. Joint effusion of the middle carpal joint was graded subjectively from 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe) with the limb weightbearing. The single experienced examining veterinarian performed a reaction to passive carpal flexion by positioning MC3 lateral to the radius and parallel to the ground and applying force for 30 seconds. A subjective grading was then given from 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe) based on the degree of reaction elicited by the horse and the resistance to carpal flexion. No objective lameness evaluation was performed on any of the horses in this study.

Radiographs were assessed by a registered specialist in equine surgery blinded to the study. Since fractures or osteochondral fragmentation cases were excluded from the study, the radiographs were scrutinized to mainly detect radiographic signs associated with repetitive trauma to the articular cartilage and subchondral bone plate resulting in middle carpal joint osteoarthritis. Therefore, sclerosis or lysis, enthesiopathy, and osteophytosis of the carpal bones forming the middle carpal joint were graded using a subjective grading system (**Table 1**). As these findings may represent different aspects or phases of carpal joint disease, it was decided to select the highest grade of any category as the horse's overall grading. Sclerosis and lysis were graded as follows: None or mild sclerosis and no lysis = 0, moderate sclerosis and/or mild lysis = 1, severe sclerosis and/or moderate lysis = 2, any degree of sclerosis and severe lysis = 3. Enthesiopathy was graded as follows: None = 0, mild = 1, moderate = 2, severe = 3. Osteophytosis was graded as follows: None = 0, mild = 1, moderate = 2, severe = 3. All carpal structures were assessed for completeness and to ensure there was no confounding findings from clinically significant radiological abnormalities in areas other than the middle carpal joint.

2.3. Group Allocation and Blinding

Horses were allocated into the three treatment groups in sequence of enrolment by a computer-generated random sequence table. The trainer was blinded to the treatment groups. The examining veterinarian performed the initial assessment and all repeat examinations and was unaware of horse's name, treatment, or previous findings. The treating veterinarian, different from the examining veterinarian, was presented the horse on the day after initial assessment for treatment. An independent third party performed all statistics in a blinded fashion and the study endpoint would be determined when statistical significance was shown to exist.

2.4. Treatment

The treating veterinarian administered one of three treatments in the middle carpal joint; 50 mg (2 mls) of 2.5% PAAG (Arthramid Vet)[2], 12 mg (2 mls) of TA (Triamolone-Forte)[3] or 20 mg (2 mls) of sodium hyaluronate (HA) (Hyonate)[4] (with two further intravenous injections of 40 mg (4 mls) at weekly intervals in the HA group only).

Table 1
Radiological scoring system of carpal radiographs.

Score	0	1	2	3
Sclerosis and/or lysis of radiocarpal, intermediate carpal or radial facet of third carpal bones	None to mild sclerosis, and no lysis	Mild lysis and/or moderate sclerosis	Moderate lysis and/or severe sclerosis	Severe lysis and any degree of sclerosis
Enthesophytes on dorsal aspect of radiocarpal or intermediate carpal bones	None	Mild - just noticeable	Moderate - sharp definition but remains discrete overall	Severe - sharp and obvious
Osteophytes (remodelling) associated with middle carpal joint	None	Mild - just noticeable	Moderate - sharp definition but remains discrete overall	Severe - sharp and obvious

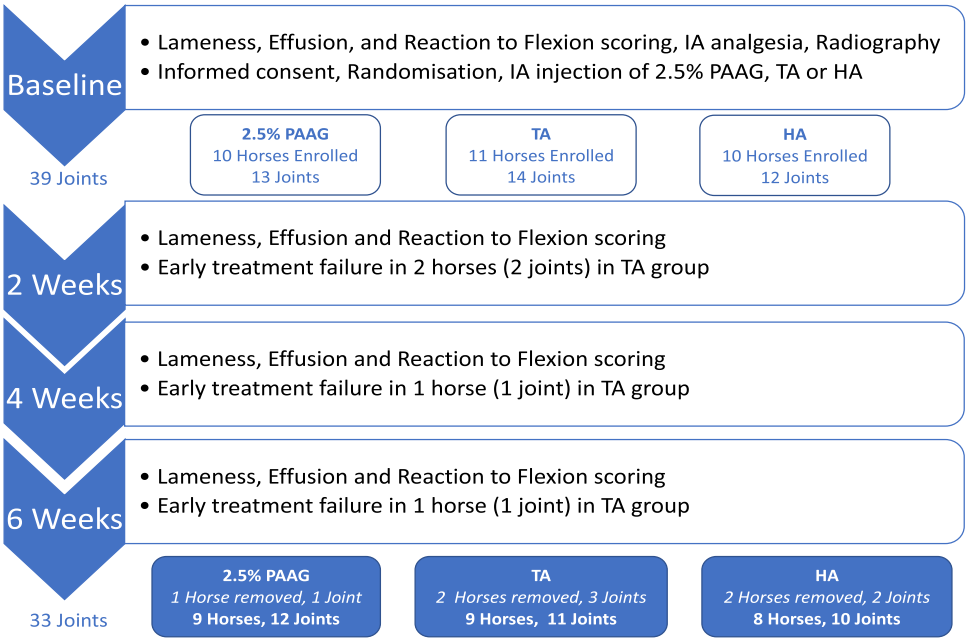

Fig. 1. Flowchart of study design and protocol. Intra-articular (IA), 2.5% polyacrylamide (PAAG), triamcinolone acetonide (TA) and sodium hyaluronate (HA).

2.5. Repeat Examinations

Repeat examinations were performed on weeks 2, 4 and 6 by the same blinded examining veterinarian at the same location, on the same surface and assessing the same study parameters (lameness, joint effusion and reaction to flexion) for all groups and, again at 12 weeks for the 2.5% PAAG treated group only. A maximum of 2 days separated the last fast work of a given horse and a repeat examination. A summary of the experimental design is available in Figure 1.

2.6. Data Analysis

No pre-study power calculations were used, and the study was designed to compare the relative efficacy of the test product to standard joint treatments (non-inferiority) and allowed for a continuation of the study until this was potentially achieved. Pre-treatment (admission) scores for lameness, joint effusion, reaction to carpal flexion, type of race the horse was trained for (sprinters were those racing under 1600 m, milers between 1600 to 2000 m and stayers over 2000 m) and age were compared between groups using non-parametric Kruskal-Wallis, Multifactor Analysis of Variance and Dunn's All Pairwise Comparison Test to ensure there were no confounding variables between groups despite random allocation. Chi-squared tests analysed radiological scores on admission.

Within each of the three parameters (lameness, joint effusion, reaction to carpal flexion) scores were reviewed over the three time points and horses allocated to 4 clinical outcomes: 'Success' was defined as a complete resolution of the variable over time to score 0; 'Nil' was defined as no change from initial examination or some improvement but without complete resolution and 'Fail' was a worsening in the variable at each assessment. Horses were considered 'withdrawn' if removed before the completion of the six-week study (by a blinded observer) due to an intolerable deterioration in lameness. Nil, fail and withdrawn were therefore grouped into an 'unsuccessful' category for statistical analysis.

Data were analyzed using Chi-Squared tests for statistical comparisons with significance set at $P < .05$ (Statistix 10.0)[5] and, point estimates for the difference in percentage of successful treatments and associated 95% Confidence Intervals.

2.7. Quality Control

This study was performed under Good Clinical Practice (GCP) standards, being independently verified and monitored by an independent Contract Research Organization (CRO). Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, monitoring, recording, auditing, analysing and reporting clinical studies evaluating veterinary products. Compliance with this standard provides assurance about the integrity of the clinical study data, and that due regard has been given to animal welfare and protection of the personnel involved in the study, the environment and the human and animal food chains.

Table 2
Pre-admission variables per treatment group.

	2.5% PAAG	TA	HA	
Number	12	11	10	*P*-values (At the 95% CI)
Age range (mean/median)	2-5 (3.08/3)	2-4 (3/3)	2-6 (2.8/3)	$P > .05$
Sprinter (%)	5 (42)	5 (45)	4 (40)	$P > .05$
Miler (%)	5 (42)	4 (36)	4 (40)	
Stayer (%)	2 (16)	2 (19)	2 (20)	
Lameness range (mean/median)	1-3 (1.67/1)	1-4 (2.18/2)	1-3 (1.8/1.5)	$P > .05$
Effusion range (mean/median)	0-2 (0.5/0)	0-1 (0.1/0)	0-2 (0.2/0)	$P > .05$
Reaction to carpal flexion range (mean/median)	0-2 (0.67/0.5)	0-2 (0.54/0)	0-2(0.6/0)	$P > .05$
Radiological grade range (mean/median)	0-2 (0.5/0)	0-2 (0.36/0.5)	0-2 (0.6/0.5)	$P > .05$

3. Results

3.1. Treatment Groups

A total of 31 horses (39 joints) were enrolled with an age range of 2 to 6 years and a mean of 3 years (Table 2). Overall, no significant differences between treatment groups for the following pre-admission variables were noted: age, type of race the horse was trained for (sprinter, miler, stayer), radiographic scores, joint effusion, reaction to flexion, or lameness grade ($P > .05$).

None of the horses developed any adverse reactions to any treatment.

A total of 5 horses (6 joints) were removed during the study on request of the owners for reasons unrelated to treatment and were excluded from the statistical analysis (Fig. 1).

The 2.5% PAAG group was initially made up of 10 horses, of which 3 horses had both middle carpal joints (left and right) affected. One horse was removed from the study (1 joint affected only), resulting in 12 middle carpal joints for treatment evaluation. There were initially 11 horses enrolled in the TA group, of which 3 horses had both middle carpal joints (left and right) affected. Two horses were removed (1 horse with both joints affected, 1 horse with one joint affected), resulting in 11 middle carpal joints for treatment evaluation. There were initially 10 horses enrolled in the HA group, of which 2 horses had both middle carpal joints (left and right) affected. Two horses were removed (1 joint affected each), resulting in 10 middle carpal joints for treatment evaluation. A total of 26 horses (33 joints) were then available for treatment analysis at the end of the study.

3.2. Horses Withdrawn

In the TA group, 4 horses (4 joints; 2 at 2 weeks, 1 at 4 weeks, and 1 at 6 weeks) were withdrawn due to intolerable deterioration in lameness before completing the study period and included in the statistical analysis as treatment failures. None of the horses were withdrawn in the 2.5% PAAG or in the HA groups.

3.3. Radiological Grades on Admission

On admission, 19/33 (58%) joints were graded as 0. Five joints showed no abnormal radiographic findings (2 in each of the 2.5% PAAG and TA groups and 1 in the HA group) and 14 joints showed mild sclerosis of the radial facet of C3 and/or mild sclerosis of the radiocarpal or intermediate carpal bone (6 in 2.5% PAAG group, 5 in TA group, and 3 in HA group) (Table 2).

Twelve joints out of 33 (36%) were graded as 1. They showed moderate sclerosis of radial facet of C3 and/or mild remodelling of the dorsodistal aspect of the radiocarpal or intermediate carpal bone (4 in 2.5% PAAG group, 5 in TA group, and 3 in HA group).

Two joints out of 33 (6%) were graded as 2. They showed moderate sharp osteophytic remodelling of the dorsodistal aspect of the radiocarpal bone with associated moderate subchondral sclerosis (1 in 2.5% PAAG group and 1 in HA group).

3.4. Lameness

At 2-weeks, the percentage and number of lame free joints was 50% for 2.5% PAAG (6/12), 27% for TA (3/11) and 40% for HA (4/10), with no statistically significant difference between groups ($P = .537$).

At 4-weeks, it was 83% for 2.5% PAAG (10/12), 36% for TA (4/11), and 60% for HA (6/10), with a statistical difference ($P = .042$) between groups in favor of the 2.5% PAAG treated group.

At 6-weeks (Table 3; Fig. 2), it was 83% for 2.5% PAAG (10/12), 27% for TA (3/11) and 40% for HA (4/10) with again a statistical difference ($P = .019$) between groups in favor of the 2.5% PAAG treated group.

Pairwise comparisons (Table 3) indicated a significantly higher success rate for lameness in the 2.5% PAAG group versus TA ($P < .05$; difference = 56%, 95%CI 22–90%) and versus HA ($P < .05$; difference = 43%, 95%CI 6–80%).

Table 4 summarizes lameness results at 6-weeks with the exclusion of bilaterally lame horses. Treatment effects of 100%, 14% and 33% were observed for 2.5% PAAG, TA and HA, respectively. Differences in effect of 86% (95% confidence interval 60–100%) and 67% (13–100%) were observed for 2.5% PAAG versus TA and 2.5% PAAG versus HA, respectively; $P = .003$ and 0.014 for the respective comparisons.

Of the joints treated with the 2.5% PAAG that were lame free at the 6-weeks point (10/12), all remained lame free at 12-weeks, and all continued in full training.

3.5. Joint Effusion

At 2-weeks, the percentage and number of successful cases for the joint effusion parameter was 25% for 2.5% PAAG (3/12), 0% for TA (0/11) and 0% for HA (0/10).

At 4-weeks, it was 50% for 2.5% PAAG (6/12), 0% for TA (0/11), and 0% for HA (0/10).

At 6-weeks (Table 3; Fig. 3), it was 50% for 2.5% PAAG (6/12), 0% for TA (0/11) and 0% for HA (0/10), with a statistical difference ($P = .002$) between groups in favor of the 2.5% PAAG treated group. Pairwise comparisons indicated a significantly higher success rate for joint effusion in the 2.5% PAAG group versus TA ($P < .05$; difference = 50%, 95%CI 22-78%) and versus HA ($P < .05$; difference = 50%, 95%CI 22–78%).

Table 3
Results at 6-weeks for lameness, effusion, and reaction to carpal flexion of the joints treated with 2.5% PAAG, TA or HA.

Treatment	2.5% PAAG	TA	HA	Total	Chi-Squared Statistic	*P*-Value
LAMENESS						
Success	10*	3	4	17		
Nil	1	1	3	5		
Fail	1	3	3	7		
Withdrawn	0	4	0	4		
Proportion Successful Treatments	83.3%*	27.3%	40.0%		7.98	**.019**
EFFUSION						
Success	6*	0	0	6		
Nil	3	5	6	14		
Fail	3	2	4	9		
Withdrawn	0	4	0	4		
Proportion Successful Treatments	50.0%*	0.0%	0.0%		12.83	**.002**
REACTION TO FLEXION						
Success	6	1	2	9		
Nil	4	3	6	13		
Fail	2	3	2	7		
Withdrawn	0	4	0	4		
Proportion Successful Treatments	50.0%	9.1%	20.0%		5.22	.073
PAIRWISE COMPARISONS	Point Estimate of Difference (%)	Lower 95% CI (% Difference)	Higher 95% CI (% Difference)	Chi-Squared Statistic	*P*-Value	
LAMENESS						
2.5% PAAG vs. TA*	56.1%	22.3%	89.8%	7.34	**.0007**	
2.5% PAAG vs. HA*	43.3%	6.4%	80.3%	4.43	**.035**	
TA vs. HA	-12.7%	-52.9%	27.5%	0.01	.944	
EFFUSION						
2.5% PAAG vs. TA*	50.0%	21.7%	78.3%	7.44	**.014**	
2.5% PAAG vs. HA*	50.0%	21.7%	78.3%	6.88	**.015**	
TA vs. HA	0.0%	-30.1%	30.1%	0	1	
REACTION TO CARPAL FLEXION						
2.5% PAAG vs. TA	40.9%	7.9%	73.9%	4.54	**.069**	
2.5% PAAG vs. HA	30.0%	-7.6%	67.6%	2.12	.204	
TA vs. HA	-10.9%	-41.0%	19.1%	0.51	.587	

Success, complete resolution; Nil, no change or partial improvement; Fail, deterioration of variables from the time at admission; Withdrawn, those withdrawn from the study before 6 wk due to treatment failure and considered as Fail.
* An asterisk (*) indicates statistical differences when $P < .05$.

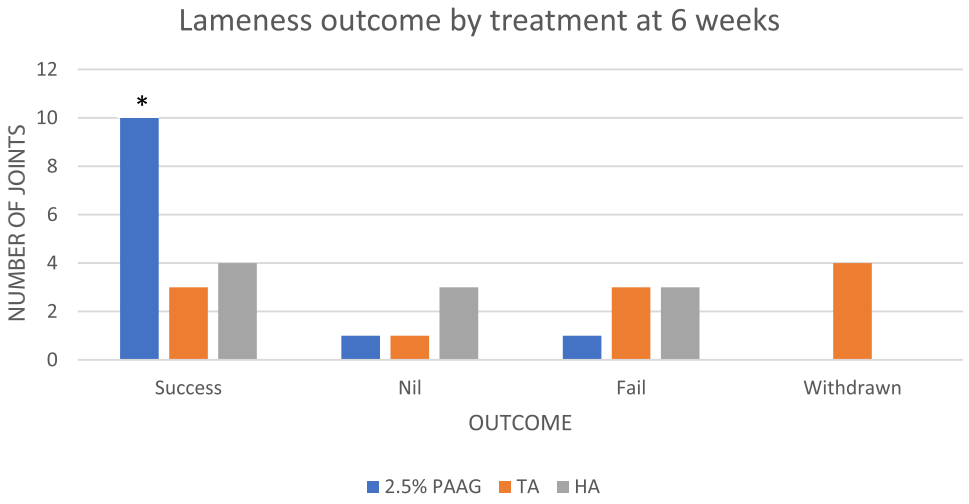

Fig. 2. Effect of 2.5% PAAG, TA and HA on lameness at 6 weeks post-treatment. Y axis = number of joints. X axis = outcome per treatment group: Success = complete resolution, Nil = no change or partial improvement, Fail = deterioration of variables from time of administration, and Withdrawn = those removed from the study before 6 weeks for treatment failure and results included in the analysis. Significant difference ($P < .05$) between treatment groups is indicated as an asterisk (*).

3.6. Response to Flexion Test

At 2-weeks, the percentage and number of successful cases for the reaction to flexion test parameter was 25% for 2.5% PAAG (3/12), 18% for TA (2/11), and 30% for HA (3/10).

At 4-weeks, it was 42% for 2.5% PAAG (5/12), 9% for TA (1/11), and 20% for HA (2/10).

At 6-weeks (Table 3; Fig. 4), it was 50% for 2.5% PAAG (6/12), 9% for TA (1/11) and 20% for HA (2/10), with no statistical difference between groups ($P = .073$).

Table 4
Results at 6-weeks for lameness of joints treated with 2.5% PAAG, TA or HA; Bilaterally lame horses excluded.

Treatment	2.5% PAAG	TA	HA	Total	Chi-Squared Statistic	*P*-Value
LAMENESS						
Success	6*	1	2	9		
Nil	0	1	2	3		
Fail	0	1	2	3		
Withdrawn	0	4	0	4		
Proportion Successful Treatments	100.0%*	14.3%	33.3%		10.21	**.006**
PAIREWISE COMPARISONS	Point Estimate of Difference (%)	Lower 95% CI (% Difference)	Higher 95% CI (% Difference)	Chi-Squared Statistic	*P*-Value	
LAMENESS						
2.5% PAAG vs. TA*	85.7%*	59.8%	100.0%	9.55	**.002**	
2.5% PAAG vs. HA*	66.7%*	13.3%	100.0%	6.00	**.014**	
TA vs. HA	-19.0%	-65.0%	26.9%	0.66	.416	

Success, complete resolution; Nil, no change or partial improvement; Fail, deterioration of variables from the time at admission; Withdrawn, those withdrawn from the study before 6 wk due to treatment failure and considered as Fail.
* An asterisk indicates statistical differences when $P < .05$.

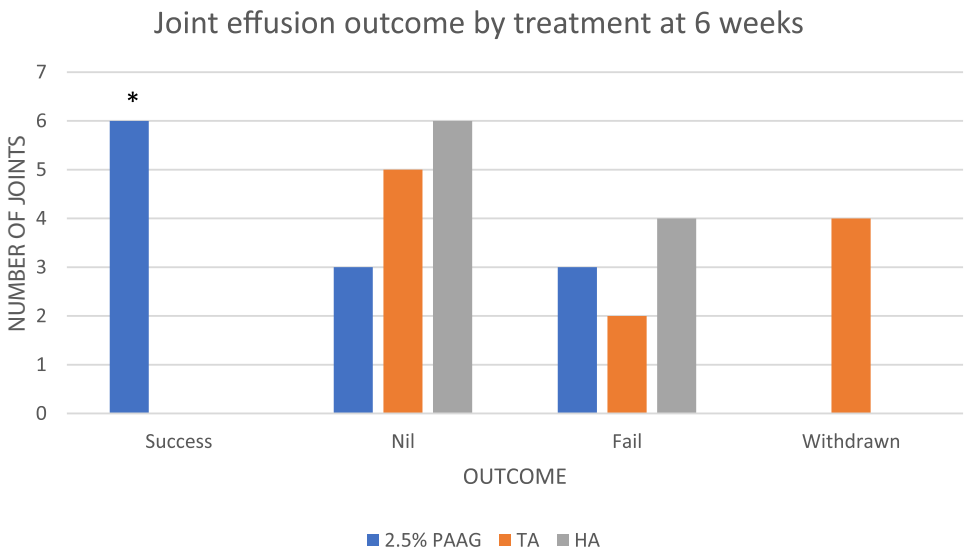

Fig. 3. Effect of 2.5% PAAG, TA and HA on joint effusion at 6 wk post-treatment. Same legend as for Figure 2.

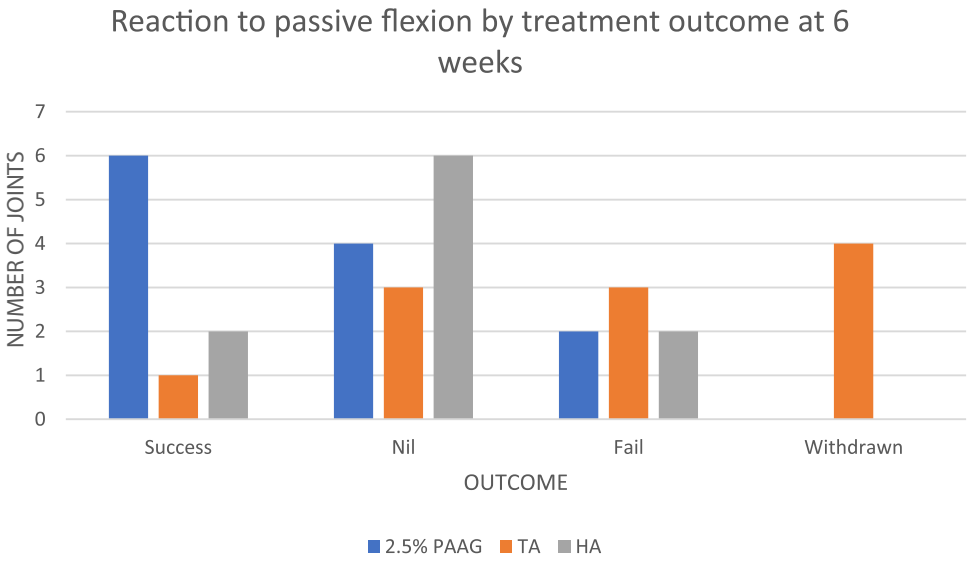

Fig. 4. Effect of 2.5% PAAG, TA and HA on reaction to flexion at 6 wk post-treatment. Same legend as for Figure 2.

3.7. Summary

Treatment results at 6 weeks are summarised in Table 3. Overall, there was no statistical difference between any of the groups at 2-weeks. By 6-weeks, a significant reduction in lameness and joint effusion with 2.5% PAAG was noted. There was no significant difference in outcomes between the TA and HA groups at any time point.

4. Discussion

This study showed a significantly better response of 2.5% PAAG when compared to conventional treatments (TA and HA) for the management of naturally-occurring middle carpal joint disease over a 6-weeks period. Prolonged activity of 2.5% PAAG up to 12 weeks was also noted. Middle carpal joint disease (osteoarthritis), characterized by lameness, joint effusion and pain on flexion, is the most common joint pathology encountered at the training facility participating in the study, and often requires repeated treatments with conventional therapies such as TA and HA [11], resulting in a significant number of days lost to training.

The study investigated a naturally-occurring disease, as it was more applicable in the clinical setting than a carpal chip model. The results of the pre-admission variables (Table 2) indicate that the severity of disease was relatively mild on admission and was likely the result of synovitis/capsulitis or early osteoarthritis [12] more than advanced osteoarthritis. Thirty one out of 33 joints (94%) had no or only mild radiographic changes (grade 0-1), indicative of repetitive cartilage and subchondral bone trauma and secondary osteoarthritis. In contrast, previous studies of 2.5% PAAG [5–8] had cases with more advanced osteoarthritic changes, and with a majority having had previous intra-articular medications over a prolonged period before medication with 2.5% PAAG. The breed, age, athletic use of the horses and treated joints were also different in previous studies investigating the effects of 2.5% PAAG [5–8]. The horses were older, only 8/115 were racing Thoroughbreds and just 3/115 of the treated joints were carpal joints. Our double-blinded randomized study suggests that 2.5% PAAG may be indicated earlier in the naturally-occurring disease process than previously thought, and as proposed in a more recent study in racing Thoroughbreds [9].

The outcome measure of lameness was considered the primary clinical parameter in our study as it would be the primary presenting complaint and resolution of lameness the primary goal of treatment. The 2.5% PAAG treated group showed a statistically stronger improvement in lameness scores ($P < .05$) when compared to both TA and HA treated groups at 4 and 6 weeks with 83% of the joints lame free. These results are consistent with previous studies of 2.5% PAAG [5–9].

Our study used positive controls, and a statistical difference was noted between treatments from 4 weeks post-admission, indicating a superior relative efficacy of 2.5% PAAG versus TA or HA by that time point. Previous studies have also shown that 2.5% PAAG takes between 2 to 4 weeks to have an effect [5–9], due to its proposed mechanism of action [13]; 2.5% PAAG takes between 2 to 4 weeks to integrate and increase the elastic resistance of osteoarthritic-affected joint capsules through deposition of collagen fibres into the intima/sub-intima of the synovium. This leads to a reduction and then stabilization of osteoarthritic changes, as detected by magnetic resonance imaging, returning the joint capsule elasticity to a pre-diseased state and increasing functionality to withstand the forces placed upon the joint during locomotion [14]. Recent human research investigating 2.5% PAAG in the management of femorotibial joint osteoarthritis also showed statistically significant improvement in joint stiffness, pain and functionality from 4-weeks after intra-articular administration [15]. Our findings support that a 4 to 6-week period is necessary before clinicians should draw any quantifiable conclusions relative to a joint's response to 2.5% PAAG treatment.

A reduction in effusion and reaction to flexion was noted in 50% (6/12) of the joints treated with 2.5% PAAG in our study. Although not as marked at 6 weeks, this effect was present and consistent with what is reported elsewhere [7,9]. It is known that upon injection into joints, 2.5% PAAG adheres to the synovial lining through its ability to exchange water molecules [1–3]. This could immediately act to reduce exposure of synoviocytes to pro-inflammatory cytokines in the inflamed or diseased joint, but this mechanism of action remains to be proven. It is also known that mononuclear cells infiltrate the synovium in response to 2.5% PAAG [4]. Wehling *et al.* (2007) showed that mononuclear cells respond to a pyrogenic-free substance, such as 2.5% PAAG, with the release of anti-inflammatory cytokines interleukin-1 receptor antagonist protein, transforming growth factor-β1, and insulin-like growth factor 1, amongst other cytokines [16]. This may explain the reduction in joint effusion noted before augmentation of the joint capsule elastance is known to occur.

Histological studies [4] have shown that throughout 14 up to 42 days the gel becomes fully integrated into the synovial lining and its immediate surrounding tissue of the inner joint capsule by a combination of cell migration and vessel ingrowth forming a thick, cushion-like membrane consisting of vessel integrated gel covered by a hypercellular synovial cell lining facing the joint cavity. As a result, 2.5% PAAG has a long-lasting augmentation effect on both the joint capsule and synovium by increasing the capsule's elasticity and tensile strength and improving its capacity to transfer load [13]. A more recent *in vitro* study model demonstrated that the coefficient of friction of both damaged and native bovine cartilage was lowered by almost 60% after exposure to PAAG hydrogel, with histological samples showing retention and localization of the gel on the cartilage surface [17]. Samples were tested seven days after exposure to the hydrogel and demonstrated a potential lubricating ability, at least in the early stages after treatment [17].

Other speculative mechanisms of actions could include that augmentation and cushioning of the synovial membrane may increase the threshold for mechanoreceptor and nociceptor activation in the capsule itself, disrupting the cycle of hypersensitization characteristic of synovitis [18]. We also consider whether forming a new and hypercellular synovial cell lining may improve the nature of synovial fluid within the joint itself. These properties would reduce the pain and inflammation of synovitis and capsulitis, which are significant in the pathogenesis of equine OA [18]. However, half (6/12) of the 2.5% PAAG treated joints remained either effused or painful on flexion, even though they had become lame free. Clinicians using 2.5% PAAG may need to expect this to occur in a certain number of treated joints and means the exact mechanism of action still remains unclear or is multifaceted. This area should be the focus of further studies.

TA is a corticosteroid commonly used to treat non-septic lameness due to its potent anti-inflammatory properties [19]. A 12mg dose of TA administered twice, on days 13 and 27 post-surgery, in a carpal osteochondral fragmentation model, was shown to cause a clinically significant reduction in lameness [20]. The average lameness grade at day 72 post-surgery was reported as 0.63 (AAEP scale) with 3 horses (50%) perceived to be lame-free, 2 still being grade 1 and one horse being grade 2/5. Although their experimental model of joint inflammation is difficult to compare to a naturally-occurring joint disease where none of the horses had osteochondral fragments, our results with TA at 42 days with only a single 12mg injection, appear less favorable (27.3% lame-free horses in our study vs. 50% [20]). Furthermore, 4 horses were withdrawn in our TA group due to intolerable deterioration of the lameness. This suggests TA, at least at this stage of the naturally

occurring carpal joint disease, may be a poor option when significant exercise is maintained. It is also surprising that the TA treated group in our study did not show better results for joint effusion compared to the 2.5% PAAG and HA treated groups. This may reflect the low sensitivity of the subjective measure for joint effusion used in this study, timing of the examinations, and the relatively low numbers of TA cases due to early withdrawals from treatment failures.

HA is a disease-modifying agent that has shown mixed results in alleviating lameness in equine carpal osteoarthritis fragmentation models [21,22]. The number of lame-free joints at 6 weeks in our study (40%) is superior to what was noted in their studies, but our horses were examined earlier (days 14 and 28) and only out to day 42. Repeated HA doses are commonly used in clinical practice and have been extrapolated from equine experiments [21,22]. Interestingly, although not statically significant, HA appeared to perform better than TA in our study. This was not expected and might be explained by the fact the horses enrolled in our study were mildly affected, giving a chance for a disease-modifying drug to alter the course of the naturally-occurring disease and subsequently improve clinical symptoms, at least in the short term. Testing the combination of HA and TA could have been interesting, as this represents a common practice in the equine industry, although the efficacy of this combination has recently been questioned versus TA alone [23].

Forty-eight hours of box rest post-injection was used as it allows sufficient time for TA and HA to penetrate the synovial structures, reduces clearance rates, reduces the risk of hemarthrosis post-injection, and is what is commonly adopted in clinical practice [24]. The same box resting period was implemented in the 2.5% PAAG group. Previous studies of 2.5% PAAG had varying resting periods, with anywhere from 2 weeks hand walking and 2 weeks of small paddock turn out [5–7], to 5 days box rest with 3 weeks hand walking [8], all followed by an increasing exercise regime. This does not match trainers and owners' expectations in a racing environment and a criticism of the previous studies investigating the effect of 2.5% PAAG is that rest alone may have alleviated the lameness. In our study, the trainer was blinded to the treatment groups so that a continuation of galloping exercise was the expectation of the connections involved, unless the horse was unable to perform galloping exercise due to lameness or sickness. In those cases, they were withdrawn (and entered as a treatment failure; 4 horses) or excluded from the study analysis (5 horses with illness or injury unrelated to treatment and excluded from the analysis). Despite the relatively short period of rest used in our study, the relative efficacy of the 2.5% PAAG was unaffected and showed superiority to the other conventional treatments. This again may indicate 2.5% PAAG is a better option for early treatment intervention, especially in the face of ongoing exercise regime.

Several study limitations were apparent and include a relatively low numbers of horses. Pre-study power calculations were not used but the study design allowed for the study's continuation until a statistically significant effect was achieved. This end point was determined by an independent statistician remote to the study. Under our legislation, animal ethics guidelines state there is no reason for continuing a study once statistical significance is achieved so a larger sample size was not included.

Outcome parameters, although based on clear scoring systems, were subjective. Lameness evaluation by an experienced equine veterinarian is subjective in nature, with inertial sensor systems (ISS) and force platforms (FP) providing objective data. However, it has been shown that the percentage of horses identified as lame in an osteochondral fragment model, independent of time, was the highest with ISS (60%) followed by blinded subjective evaluation (51%) and force platforms (42%), and that the difference between subjective evaluation and inertial sensor systems was not statistically different [25]. Blinded subjective evaluation and the ISS agreed which forelimb was lame more often (50%) 25. Using a well-defined lameness scale (AAEP) in this study was also more applicable in the clinical setting and the measure of outcome success was being 'not lame', which enabled the single examining veterinarian to detect large and significant lameness changes. It was also suitable for showing statistical differences between the treatment groups to assess efficacy in a naturally-occurring disease model.

Horses with bilateral lameness were included in our study as this was a common presentation in the clinical setting. From a clinical perspective, horses with bilateral forelimb lameness will also present with bilateral joint distention, bilateral joint heat, and bilateral reaction to joint manipulation (flexion test and digital pressure), and typically one limb will be lamer than the other. All limbs had to respond to intraarticular analgesia of the middle carpal joint to enter into the study. Although follow-up examinations could have included repeating the intraarticular analgesia, this could in itself adversely affect the results by altering the joint fluid environment or causing iatrogenic trauma and was therefore not considered in a clinical setting. Further statistical analysis was performed however to additionally evaluate what effect the exclusion of bilaterally lame horses would have. Although this resulted in relatively low numbers of joints included in each group (2.5% PAAG = 6, TA = 7, HA = 6), there was still a significant difference in treatment effect in favor of the 2.5% PAAG treated group compared to both TA and HA.

Assessment of effusion was also subjective. It is still surprising that neither the TA nor HA groups showed any significant reduction in effusion at any time point. This may be a further indication of the insensitivity of the methods used, the lack of significant effusion pre-treatment, possibly due to the early stage of the disease in the horses used in the study, related to the timing of the examinations after treatment, or a lower response than expected with conventional therapies in the face of ongoing exercise. Although less than ideal, the same subjective scores were used by a single blinded and experienced examining veterinarian for all horses and did allow detection of a statistically significant difference between 2.5% PAAG and the TA/HA treatment groups at 4 and 6 weeks ($P < .05$) and is consistent with previous studies of 2.5% PAAG [7,9]. Objective data measurement using ultrasound or measuring joint circumference with a measuring tape may have allowed detection of more subtle differences between the TA and HA groups and at other time points.

Assessing changes in the quality of synovial fluid could also have provided more objective measures of joint health and may be a useful focus of future studies of 2.5% PAAG. A reliable biomarker for joint fluid health is not readily available although direct measurement of synovial fluid viscosity both before and after treatment has been used in a recently published study assessing the use of equine mesenchymal stem cells as a treatment for OA [26]. Other studies on TA and HA [18,19] have also investigated the quality of synovial fluid using laboratory parameters and histological examinations. An *antemortem* synovial biopsy technique is now available for equine use in a non-clinical setting [27]. The authors suggest further research should include both synovial fluid viscosity assessments and synovial biopsies. These would also be useful to investigate any potential disease-modifying effects of the 2.5% PAAG.

Quantifying radiographic pathology to ensure a lack of bias across treatment groups was difficult. No standardized radiographic scoring system was available to the authors' knowledge so one was constructed *post hoc* for the purpose of this study. Most cases had none (19/33) or only mild (12/33) radiographic changes indicative of repetitive exercise-induced trauma to the cartilage and subchondral bone plate associated or not with sec-

ondary osteoarthritic changes. Although potential bias between groups may have been mitigated by blinding and randomization and, considering no statistically significant difference was noted between groups at enrolment, this may have been due to the relatively low numbers in the study. The TA treated group did have a slightly lower, although not statistically significant, radiographic grade mean (0.36) compared to the 2.5% PAAG (0.5) and HA (0.6) treated groups. This random allocation could potentially have impacted the results. Hence, we ensured using several appropriate statistical tests (see materials and methods), there was no reason to believe the small variations recorded between treatment groups on admission could affect the treatment outcome. The radiographs accurately reflected the population of horses typically seen in the racing environment where the study took place and, the relative insensitivity of radiographs to detect early and small changes in early naturally-occurring disease [12] and the slow progression of OA are known to be problematic in clinical trials [7].

In the case of none or mild radiographic findings with a positive result to intra-articular anaesthesia, synovitis/capsulitis was the most likely differential diagnosis. Acute synovitis and capsulitis are now recognized as the most common problem in equine athletes' high motion joints and contribute to the degradative process in articular cartilage by releasing catabolic enzymes, inflammatory mediators, and cytokines [15]. The fact that 2.5% PAAG was superior to conventional treatment groups with relatively low numbers and in early stage naturally-occurring OA appears to offer clinicians a valuable alternative to managing cases proactively and earlier on in the disease process. Any attempt to modify the degradative cycle of equine OA early on, and without the possible risks associated with TA [28], appears worthwhile.

5. Conclusions

In conclusion, 2.5% PAAG treatment led to a statistically significant better outcome in the management of middle carpal joint lameness in Thoroughbred racehorses in full training affected by naturally-occurring joint disease when compared to conventional pharmaceuticals TA and HA. This double-blinded clinical trial showed that 83% of the joints treated with 2.5% PAAG and presented with a lameness grade ranging from 1-3/5 (AAEP scale) and from no to moderate radiologically-detectable osteoarthritic changes, became lame free at 4 weeks, with persistent results at 6 and 12 weeks. In contrast to earlier studies [5–8], these results indicate that 2.5% PAAG may be worth considering early on instead of late, or as a last option, in the course of naturally-occurring carpal synovitis or capsulitis in the racehorse.

6. Manufacturer's Addresses

1. Mepivacaine Injection: CEVA Animal Health Pty Ltd, Glenorie, NSW, Australia, 2157.
2. Arthramid Vet: Contura, 2860 Soeburg, Denmark.
3. Triamolone-Forte: Jurox Pty Ltd, Rutherford, NSW, Australia, 2320.
4. Hyonate: Merial Australia Pty Ltd, Macquarie Park, NSW, Australia, 2113.
5. Statisitx 10.0, Analytical Software 2013.

Author Contributions

Leigh Travis de Clifford: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – Original Draft and Supervision. Jason Nicholas Lowe: Conceptualization, Methodology, Validation, Formal analysis, Writing - Review & Editing, Project administration and Funding acquisition. Campbell Duirs McKellar: Investigation, Writing - Review & Editing. Catherine McGowan: Formal analysis, Writing - Review & Editing and Supervision (supervised Leigh Travis de Clifford in analysis and write-up of the project for his Masters in Veterinary Professional Studies, University of Liverpool). Florent David: Conceptualization, Methodology, Validation, Formal analysis, Writing - Review & Editing, and Project administration.

Financial Disclosure

This work was supported by Innovative Medical Solutions Limited. The company provided the investigational veterinary products (2.5% PAAG) and control products (triamcinolone acetonide and sodium hyaluronate) and made a financial contribution to diagnostic procedures performed by examining and treating veterinarians during the study's conduct.

Data Accessibility Statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Owner Consents

Agent's/ owner's gave informed written consent for all horses enrolled in the study.

Study Monitoring/ Quality Control

This study was performed under Good Clinical Practice (GCP) standards, being independently verified and monitored by a Contract Research Organization (CRO), Invetus Pty Limited, Australia.

Acknowledgments

Ms. Catherine Coleman – for recording all data entry. Dr. Andrea M. Ritmeester, Dipl. ACVS of Matamata Veterinary Services - for initially assessing all radiographs taken. Ms. Sarah Johnson from the Education and Research Department of the Equine Veterinary Medical Center, a Member of Qatar Foundation – for assistance with article submission.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jevs.2021.103780.

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