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Clinical Studies

Correlation Between Acupuncture Point LI-18 and PC-1 Sensitivity and Front Hoof Surface Temperature in Horses Using Infrared Thermography

Shu Dai*, Deng-Shan Shiau, Jose Zilberschtein

ABSTRACT

The objective of this study was to determine whether acupuncture point sensitivity to palpation at LI-18 and PC-1 acupoints in horses was correlated with a difference in surface temperature between front hooves measured by high-resolution infrared camera. Client-owned horses (n=41) presenting for traditional Chinese veterinary medicine (TCVM) exams for a variety of medical issues were recruited for convenience sampling. After 20 minutes of stall rest, bilateral front hoof thermography was performed in a designated stall. This was followed by bilateral acupoint scan (APS), where the outcome was considered positive (APS^+) when an acupoint palpation, performed with an acupuncture needle guide tube (28 gauge, 2 inch), elicited a flinch response, or negative (APS^-) when the palpation equaled no reaction. Based on the APS results, horses were grouped into 1) bilateral APS^- outcome (bi-APS^-) or 2) unilateral APS^+ outcome (uni-APS^+). Within each of the 2 groups mean temperature difference between front hoof surfaces was compared. The results showed that the bi-APS^- group thermographic readings were not significantly different (p=0.551) between front hooves; however, the hoof temperatures were significantly different from each other in the uni-APS^+ group (p=0.039). Study findings suggest that a horse's APS sensitivity at LI-18 and/or PC-1 are correlated with thermographic imaging temperature of the front hooves. Further investigation and confirmation of these findings is needed along with further studies associating this temperature difference with hoof disorders for diagnostic investigations.

Keywords: acupoint scan, acupuncture, acupuncture point scan, hoof temperature, infrared camera, thermography

ABBREVIATIONS: AP: acupuncture; APS: acupuncture point scan/acupoint scan; APS^+: acupoint scan elicits a flinch response; APS^-: acupoint scan elicits no response; bi-APS^-: acupoint scan elicits no response on both sides of the body; EAC: Equine Acupuncture Center; IRT: infrared thermography; ROI: region of interest; TCVM: traditional Chinese veterinary medicine; TCM: traditional Chinese medicine; uni-APS^+: positive acupoint scan on 1 side of body only; PC-1: Pericardium 1 (acupoint); LI-18: Large Intestine 18 (acupoint); SI-16: Small Intestine 16 (acupoint); TH-15: Triple Heater 15 (acupoint); BL-14: Bladder 14 (acupoint); BL-42: Bladder 42 (acupoint)

Lameness is one of the most common health problems in horses, and locating the source can be a diagnostic challenge for equine practitioners. A national study reported that an estimated 50% of horse operations with 3 or more horses have 1 or more lame horses annually, and on a given day as many as 5% of the horses could be expected to be lame.[1] Limb or joint problems account for half of all lameness cases in the spring/winter and foot problems account for half of all cases that occur in the summer.[1] In many cases, lameness diagnosis may require advanced or comprehensive procedures such as local nerve blocks, arthroscopy, ultrasound, radiographs or magnetic resonance imaging (MRI). Some of these diagnostic tools, however, may not be readily available at many equine clinics, making diagnosis difficult.

Meridian theory is one of the core components of traditional Chinese medicine (TCM) and traditional Chinese veterinary medicine (TCVM). In Meridian theory, each of the 12 regular Meridians consists of a network that connects specific *Zang-fu* organs internally with the associated joints, limbs and body surfaces externally through a group of acupoints.[2,3] Sensitivity at specific groups of acupoints is considered highly correlated with

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disorders originating at various local and referred body sites associated with the Meridians/Channels.[4] Based on this theory, the acupuncture point scan (APS) is a method of palpating specific acupuncture points with light pressure to detect pain/sensitivity. The pattern of sensitivity has been used to diagnose and localize lameness and other disorders in horses.[5] The APS diagnostic method is not only safe and non-invasive but also without the additional cost of special equipment, and its outcomes have correlated with those of conventional clinical diagnosis.[6-11] Despite these findings, to date, this correlation has not been established using location-specific, objective and quantitative measurements. Once APS sensitivity can be correlated with a well-accepted physiologic measurement of lameness or hoof disorders, APS can be considered an evidence-based diagnostic and monitoring tool for equine lameness management.

Thermography is an imaging technique that uses an infrared camera to map and measure body surface temperature of specific anatomic sites. Skin temperature is largely under the control of the autonomic nervous system and inflammatory processes. Sagittal symmetry of thermal distribution is expected throughout the body. Asymmetric infrared emission of 1°C or greater can be indicative of sympathetic nervous system dysfunction or other pathology. The use of thermography, as a noninvasive method to detect and assess equine lameness, continues to increase along with protocols to minimize artifacts that have been associated with past use of the procedure.[12-14] Additionally, clinical investigations have demonstrated its reliability when used for diagnosing tendon-ligament injury, joint disease, stress fractures, navicular disease and caudal back pain.[14-23] Higher therapeutic success rates have been obtained when the physiological diagnostic imaging of thermography is paired with the anatomical imaging of ultrasound and radiographs for equine limb pathology.[13] With consideration of its use in lameness diagnosis, thermography can serve as a quantitative physiologic measurement for validation of acupuncture point sensitivity as a diagnostic tool.

The purpose of this study was to determine whether acupoint sensitivity has a correlation with hoof temperature derived from thermographic images. The acupoints LI-18 (Large Intestine 18) and PC-1 (Pericardium 1) have been used as key diagnostic points for front hoof pain/ disorders.[9] Both acupoints are on Meridians that flow through the front hooves with the LI Meridian starting at the craniomedial coronary band while the PC Meridian ends on the palmar side of the foot between the bulbs of the heel.[24] Sensitivity at these acupoints in TCVM indicates disorders in the front foot on the ipsilateral side of the horse and has been validated in recent studies.[9,10] Hoof pain/inflammation often is associated with regional blood circulation changes that could lead to surface thermal changes. It was hypothesized that horses without acupoint sensitivity (at LI-18 or PC-1) on either side would have no significant difference in thermal data between front hooves, and that those with only one side exhibiting APS sensitivity (at LI-18 or PC-1) would have significant difference in thermal data between front hooves.

MATERIALS AND METHODS

Animals

All adult horses, regardless of age, sex or breed, admitted to the Equine Acupuncture Center (EAC), Reddick, Florida, USA between February 3, 2020 and May 28, 2021 for TCVM exam and acupuncture/Chinese herbal medicine therapy were eligible for the study. Horses were excluded from recruitment if the owner informed consent was not obtained. Rectal temperatures of all study candidates were measured after 20 minutes of stall rest and any horse recording over 101°F or having medications or wraps on the hoof that could not be removed were excluded, as these conditions were likely to affect the thermography measurements.

Experimental Procedure

Horses admitted to the EAC were recruited for convenience sampling participation in this phase of the research investigation. After permission for study participation, each recruited horse was individually brought to the designated imaging stall. The front hooves were visually inspected for problems (e.g. wraps, topical hoof medications). After hoof inspection, the horse was allowed to rest for at least 20 minutes to acclimatize to the thermographic imaging environment.[20] Before the hoof images were taken, the foot was placed on a wood board (Figure 1). To capture images, the infrared imaging camera[a] lens was set perpendicular to the region of interest (ROI) of the targeted hoof and focused on the hair lining the coronary band. The IRT (infrared thermography) imaging techniques were directed at defining the ROI, which was a dorsopalmar view of the front hooves. The images were first captured with grey scale to allow accurate focusing and then changed to the rainbow palette for analysis.

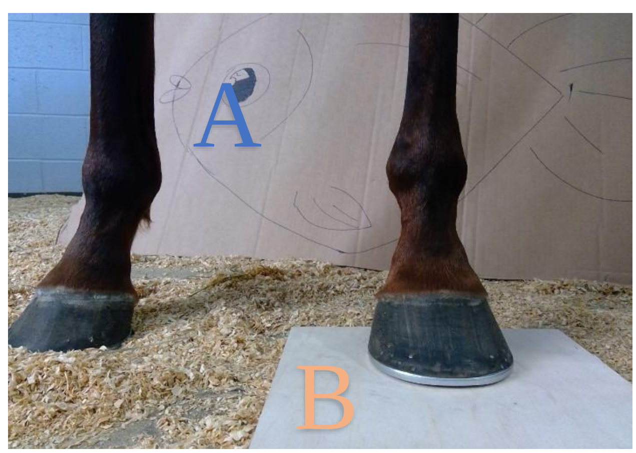

Figure 1: Before hoof images were taken, the foot was placed on a wood board (B). To ensure that nothing was in the frame that would increase camera temperature range, a cardboard screen (A) was used to block the rear legs of the horse and other objects in the stall.

Immediately after the thermo-imaging, bilateral APS was performed on LI-18 and PC-1 to determine sensitivity/ pain at each acupoint (Figure 2). The acupoint, LI-18, is located at a depression just above the jugular groove, along the line of the ventral mandible with the head extended; PC-1 is located at the depression, medial to the point of the elbow and in the fifth intercostal space. This procedure was performed using an acupuncture needle guide tube (28-gauge, 2 inch) which was stroked firmly across the skin on a neutral area of the neck (base of mane) that does not have diagnostic acupoints. This preliminary step both familiarized the horse with contact by the tool and allowed the clinician to gauge the amount of pressure to exert on the skin of that individual. The pressure was sufficient to be easily felt by the horse on the skin and underlying musculature but be well-tolerated by that individual. At LI-18, the strokes of APS were in a cranial-to-caudal direction, but at PC-1, the APS strokes were in a dorsal-to-ventral direction. The scanning stroke was repeated 5 times. To be assigned a sensitivity grade, at least 4 of 5 strokes had to have a positive response. The detailed experimental procedure followed the same protocol described in our previously reported pilot study.[25]

Figure 2: Location of LI-18 and PC-1; the long red arrow at the bottom of the picture indicates the region of interest (ROI) for thermal imaging (dorsal surface of the hoof).

DATA ANALYSIS

The thermal data, collected from the camera after the horse completed the trial, was used to calculate the mean temperature of the dorsal surface of the front hooves. A positive APS outcome (APS$^+$) was defined as the horse exhibiting sensitivity to palpation of an acupoint, whereas a negative APS outcome (APS$^-$) was defined as the horse exhibiting no response to palpation of an acupoint. Based on APS results, the hoof thermographic data sampled was organized into 2 groups: (1) bi-APS$^-$ Group (both sides had APS$^-$ outcomes); and (2) uni-APS$^+$ Group (APS$^+$ sensitivity on one side outcomes). Group thermographic data was presented as mean±SD.

Within-group and between-group statistical inferences were performed using a commercial software[b]. Within-group statistical inference tested the null hypothesis for each group, stated as no difference between hooves in mean thermal reading. Two-sided Wilcoxon signed rank test was used and the null hypothesis was rejected when the *p*-value was less than 0.05. Between-group statistical inference tested the null hypothesis that the two horse groups have the same mean between-hoof thermographic reading difference. Since the study hypothesized uni-APS$^+$ group would have greater mean inter-hoof temperature difference, the one-sided Wilcoxon rank sum test was used, and the null hypothesis was rejected when the *p*-value was less than 0.05. Horses with bilateral positive acupoint sensitivity were excluded from data analysis. To evaluate bilateral acupoint sensitivity, it would be necessary to combine thermography with knowledge of foot pain/ pathology (data not available for this study), since hoof temperature associated with bilateral acupoint sensitivity may be similarly increased due to inflammation.

RESULTS

There were 43 horses enrolled in the study. Two horses were excluded from data analysis. One horse, with behavioral issues, had a hypothermic right front leg (15.6 degrees lower than the left) without an overt clinical explanation (lameness, wounds). The other horse, diagnosed with equine protozoal myeloencephalitis, had a hypothermic left front leg (8.2 degrees lower than the right) without an overt clinical explanation for the discrepancy. Hence, there were a total of 41 horses (41 visits with no repeats of the same horse) included in the study: 27 geldings, 13 mares and 1 stallion. The study data was collected over a 1.5-year period. The age of horses ranged from 3-28 years, with an average of 10.5 years. There were 12 breeds of horses presenting to the clinic in 5 clinical categories with Quarter Horses as the predominant breed and poor performance as the most common clinical issue (Tables 1 and 2).

Out of the 41 study horses, 18 horses had a bilateral negative reaction to both acupoints (bi-APS$^-$ group) and 12 horses had unilateral APS$^+$ sensitivity (uni-APS$^+$ group). The other 11 horses had bilateral positive APS outcomes and were excluded from the analysis. Among the 18 bi-APS$^-$ horses, 9 horses had slightly greater thermographic readings on the right hoof, 6 on the left hoof, and the remaining 3 had identical readings for both hooves (Figure 3 and Table 3). The mean±SD difference was 0.21±1.55°F (p=0.551). The 12 uni-APS$^+$ horses were comprised of 8 horses with greater thermographic readings on the right hoof, 1 horse with higher thermographic readings on the left hoof, and the remaining 3 had identical readings on both hooves. The mean±SD difference was 1.06±1.63°F (p=0.039).

Table 1: Breed distribution of horses that participated in the study

Breeds	Number of Horses
American Quarter Horse	19
Thoroughbred	7
Warmblood	3
Hanoverian	3
Paint Horse	2
Percheron	1
Friesian	1
Holsteiner	1
Pony	1
Rhinelander	1
Selle Francais	1
Trakehner	1
Total	**41**

Table 2: Distribution and incidence of "reason for the visit" for a study horse to the university clinic

Reason for the Visit	Number of Horses
Poor Performance	15
Lameness	11
Internal Medicine	10
Behavior	4
Wellness	1
Total	**41**

After defining an APS outcome (+ or -) on the sensitivity to both LI-18 and PC-1 acupoints, the outcomes using only one acupoint were investigated. When only considering a horse's sensitivity to LI-18, out of the 41 study horses, 25 horses had a bilateral negative reaction (LI-18 bi-APS$^-$ group) and 11 horses had unilateral APS sensitivity (LI-18 uni-APS$^+$ group). Among the 25 LI-18 bi-APS$^-$ horses, 12 horses had slightly higher thermographic readings on the right hoof, 10 on the left hoof, and the remaining 3 had the same readings on both hooves (Figure 4 and Table 4). The mean±SD difference was 0.20±1.73°F (p=0.781). Among the 11 uni-APS$^+$ horses, 8 horses had greater thermographic readings on the right hoof, none had greater thermographic readings on the left hoof, and the remaining 3 had the same readings on both hooves. The mean±SD difference was 0.99±1.02°F (p=0.008).

When considering a horse's sensitivity to PC-1 only, out of the 41 study horses, 19 horses had a negative reaction to PC-1 (PC-1 bi-APS$^-$ group) and 13 horses had unilateral APS sensitivity (PC-1 uni-APS$^+$ group). Among the 19 PC-1 bi-APS$^-$ horses, 10 horses had slightly higher thermographic readings on the right hoof, 6 on the left hoof, and the remaining 3 had the same readings on both

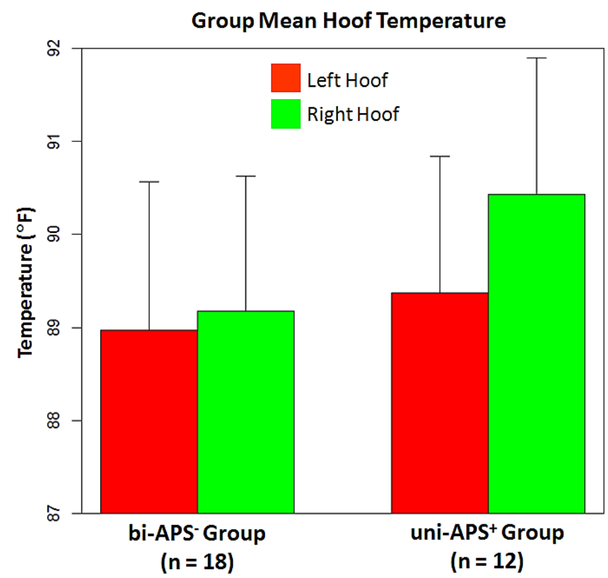

Figure 3: Mean temperature on each hoof within each APS group; with APS group based on scan sensitivity outcomes at both LI-18 and PC-1.

Table 3: Summary statistics shown in Figure 3 with between-hoof analysis

	Left Hoof	Right Hoof	L-R Difference
bi-APS$^-$ (n = 18)	88.9±6.7	89.2±6.1	0.2±1.5 (p=0.551)
uni-APS$^+$ (n = 12)	89.4±5.1	90.4±5.1	1.1±1.6 (p=0.039)

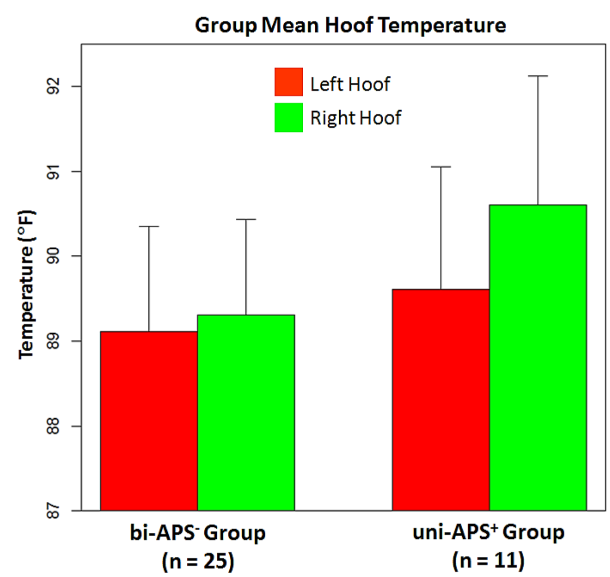

Figure 4: Mean temperature on each hoof within each APS group; with APS group based on scan sensitivity outcomes at LI-18 only.

Table 4: Summary statistics shown in Figure 4 with between-hoof analysis

	Left Hoof	Right Hoof	L-R Difference
bi-APS$^-$ (n = 25)	89.1±6.2	89.3±5.6	0.2±1.7 (p = 0.781)
uni-APS$^+$ (n = 11)	89.6±4.8	90.6±5.1	1.0±1.0 (p = 0.008)

hooves (Figure 5 and Table 5). The mean±SD difference was 0.32±1.58°F (p=0.369). The 13 PC-1 uni-APS^{+} horses, were comprised of 9 horses with greater thermographic readings on the left hoof, 1 had greater thermographic readings on the right hoof, and the remaining 3 had the same readings on both hooves. The mean±SD difference was 0.99±1.58°F (p=0.027).

When comparing the mean difference between groups, LI-18 bi-APS^{-} versus LI-18 uni-APS^{+}, findings demonstrated the mean difference in the uni-APS^{+} group was significantly greater than the APS^{-} group (p=0.042; Table 6). Comparison between the other groups, PC-1 bi-APS^{-} versus PC-1 uni-APS^{+} and bi-APS^{-} versus uni-APS^{+} (both acupoints); findings demonstrated a 3X and 5X (respectively) greater mean difference in the uni-APS^{+} than the APS^{-} group. This large mean difference, however, did not attain statistical significance for the inter-group comparison in this small study (p=0.154 and p=0.202, respectively).

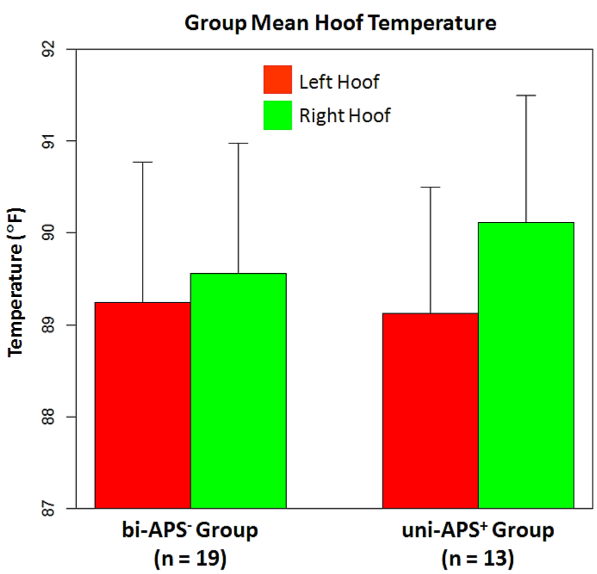

Figure 5: Mean temperature on each hoof within each APS group; with APS group based on scan sensitivity outcomes at PC-1 only.

Table 5: Summary statistics shown in Figure 5 with between-hoof analysis

	Left Hoof	Right Hoof	L-R Difference
bi-APS^{-} (n = 19)	89.2±6.7	89.6±6.2	0.3±1.6 (p=0.369)
uni-APS^{+} (n = 13)	89.1±5.0	90.1±5.0	1.0±1.6 (p=0.027)

Table 6: Inter-group comparison on L-R hoof temperature difference

	***p*-value (uni-APS^{+} > bi-APS^{-})**
LI-18 or PC-1	0.202
LI-18	0.042
PC-1	0.154

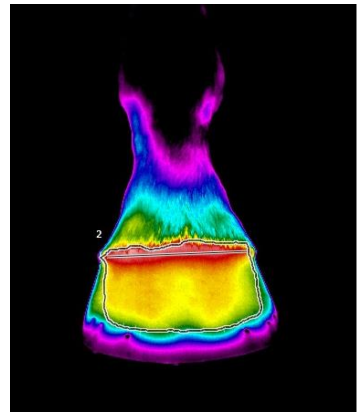

Figure 6: Region of Interest (ROI) selected for analysis; freehand drawing on the recorded images was used to select the targeted area of the hoof for data collection**.** The hand-drawn region included the hoof above the shoe nails and below the upper coronary band hair margin (hand-drawn grey line in this image).

DISCUSSION

Thermal imaging provides a quantitative measurement of body surface temperature at anatomic areas of interest through a no-contact and non-invasive process. It is a rapidly evolving technology with multiple studies investigating standardization and recommendations for clinical usefulness.[12-14] The objective of the present study was to use thermal medical imaging to determine whether acupuncture point sensitivity to palpation at LI-18 and PC-1 in horses was associated with forelimb hoof surface temperature as measured by a high-resolution infrared camera. It was hypothesized that study horses with unilateral acupoint sensitivity would have significant between-hoof thermal measurement differences, whereas those showing no sensitivity on either side would demonstrate statistically insignificant thermal measurement differences between the two hooves.

Study results showed a significant difference in between-front hoof temperature in horses with a unilateral APS positive reaction (flinches when palpated), regardless of using only one or both acupoints to define the APS outcomes. Negative APS findings on both sides of a horse, in contrast, were consistent with insignificant thermal differences between front hooves, using one or both acupoints. These results satisfied the hypothesis that horses without acupoint sensitivity (at LI-18 or PC-1) on either side would have no significant difference in thermal data between front hooves, and that those with only one side exhibiting APS sensitivity (at LI-18 or PC-1) would have significant difference in thermal data between front hooves.

When performing inter-group comparisons, the study could only conclude a statistically significant group difference (i.e. front hoof temperature difference) when using the LI-18 acupoint. This is likely because LI-18 starts at the craniomedial coronary band, which was ROI for the present study. The PC Meridian (PC-9), in contrast, ends on the palmar side of the foot between the bulbs of the heel. It is postulated that if the ROI of the study was the heel instead of a craniodorsal view; APS outcome based on PC-1 palpation, might then lead to a statistically significant group comparison conclusion similar to LI-18 in the present study. This may be an area to investigate in future studies.

Palpation of sensitive acupuncture points (equine acupoint scan) to locate pain and clinical disease is a routine procedure in equine TCVM clinical exams. Studies have demonstrated that there exists correlation between acupuncture point reactivity and lesion grade severity, which supports the APS's clinical usefulness.[8] Using medical diagnostic procedures, McCormick investigated the association between specific reactive acupoints (LI-18, SI-16, TH-15, BL-14, BL-42) and equine lameness caused by metacarpophalangeal (327 horses) or hoof (189 horses) pain.[9,10] When Channel imbalance was present (i.e. APS positive), the reactivity of an acupoint was temporarily abolished after intra-articular anesthesia of the metacarpophalangeal or distal interphalangeal joint. Similar to the present study, acupoint sensitivity was correlated with an anatomic region of interest. A limitation, however, to the McCormick studies was the ability to only correlate with pathology that had an intra-articular component. For example, subsolar abscesses, which are extra-articular, could not be correlated. The technology used in the present study mitigates these limitations as it is not only non-invasive but also detects temperature abnormalities, which is considered a more universal change associated with tissue pathology or inflammation.

There have been several thermography studies on horses, with the thermal imaging procedures/protocols being predominately focused on larger ROIs (e.g. whole extremities, the foot including the fetlock).[22-24] Based on the authors' knowledge, there has not been a standardized thermography protocol specifically for hooves in horses. Important aspects considered when developing a hoof thermography protocol include artifacts from hair, metal shoes and nails. As the hair coat on horses can significantly affect thermal imaging, the readings from images captured on the hoof surface employed in this study focused on the craniodorsal aspect of the front hooves.[13] This imaging area, which has less interference from the haircoat (only minimal coronary band hair in the image), covers almost 80% of the hoof and maximizes hoof temperature determination with little hair artifact. An additional imaging requirement to decrease variability was selecting a ROI that would not include hoof nails/shoes in a thermal reading. This would allow horses to be shod or unshod without restrictions when having hoof thermography performed. Shapes provided by computer software (i.e. various geometric outlines) did not optimally delineate the ROI area. A freehand technique to outline the ROI was developed in the pilot study to maximize the accuracy of hoof thermal measurements (Figure 6). The imaging protocol developed for this study, during the pilot study, provided additional data to help standardize equine hoof thermography. Further studies using a similar protocol would provide additional data to support the described imaging technique developed for this study.

Limitations of this study include external factors related to the horse (i.e. haircoat), which is known to affect thermal imaging.[26] This limitation was addressed by selecting an ROI centered on the hairless dorsal surface of the hooves. Ambient temperature may also affect the readings; however, based on previous studies, the external temperatures were within recommended standards.[20,27] Additionally, lack of a published standardized hoof thermography protocol to follow may have created artifacts in temperature measurements that the authors are unaware of and await further investigations to identify issues. Horses used for this study were a random population, convenience sampled upon presentation to the clinic, without pairing with diagnostic investigations. The study, therefore, was unable to correlate hoof pathology or horse breed with thermal findings. Due to convenience sampling of all eligible horses, there was a predominance of Quarter Horses in the study population. This was not evaluated for any study result effects. Study design was limited to investigating a hoof thermal association with unilateral acupoint sensitivity. To evaluate bilateral acupoint sensitivity, it would be necessary to combine thermography with foot pain/pathology (not part of the protocol), since hoof temperatures may be similarly increased due to bilateral inflammation.

The study was also limited by subject numbers, given the larger than expected variation among subjects. This resulted in the requirement of a larger sample size to derive inter-group statistical significance for 2 of the 3 comparisons made. Intergroup comparison of LI-18 uni-APS$^+$ group = 5 × LI-18 bi-APS$^-$ group and was statistically significant (p=0.042). Comparison of the other 2 groups demonstrated large group differences; uni-APS$^+$ group = 5 × bi-APS$^-$ group and PC-1 uni-APS$^+$ group ≈ 3 × PC-1 bi-APS$^-$ group, however, did not reach significance due to small sample size. Retrospectively, given the observed group difference and variation, the statistical test for group comparison would have an 80% chance (power) to conclude a statistically significant group difference if there were 60 horses in each group.

In summary, the present study suggests that APS sensitivity results at LI-18 and PC-1 are associated with thermographic imaging temperature differences between the right and left front hooves of a horse. A similar large-scale study paired with diagnostic investigation is warranted. The effect size observed in this study could serve as a guideline for sample size planning in future studies. Furthermore, future investigations could be expanded to study correlations between APS outcomes at other acupoints for intended diagnosis/treatment, and thermal changes at acupoints after acupuncture stimulation. This would further acupuncture and Meridian theory understanding and expand their evidence-based applications in veterinary medicine.

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Declaration of Interest and Funding

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this paper and the authors did not receive any specific grant of funding for authorship of this paper.

FOOTNOTES

[a] Digatherm's IR Tablet 640 thermal camera, Digatherm LLC, Ocala, FL. USA

[b] R version 3.5.2. The R Foundation for Statistical Computing, Vienna Austria; http://www.R-project.org

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