Review

Evidence-based Application of Acupuncture in Equine Practice

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ABSTRACT
Traditional Chinese Veterinary Medicine (TCVM) including acupuncture has been used to treat disease and relieve pain in horses for more than 2,000 years. A review of the current knowledge of the mechanisms of acupuncture stimulation is presented. An emphasis is placed on electro-acupuncture, which can release neurotransmitters such as 5-hydroxytryptamine (serotonin) and endogenous opioids including β-endorphin, to relieve pain. Acupuncture has been shown to be a viable integrative treatment for equine back pain, foot pain, cervical stiffness, laryngeal hemiplegia and infertility. Acupoint sensitivity on palpation may be useful for the assessment of lameness along with conventional diagnostics in horses. Future well-designed studies are needed in order to strengthen the recommendation for acupuncture in the diagnosis and treatment of clinical conditions in horses.

Keywords: acupuncture, electro-acupuncture, review, equine, pain relief, clinical practice

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ABBREVIATIONS

5-HT	5-hydroxytryptamine
ACTH	Adrenocorticotropic hormone
AP	Acupuncture points
Aqua-AP	Aqua-acupuncture
BHSL	Back half stride length
DFB	Difference front/ back
DNAP	Dry needle acupuncture
EAA	Electro-acupuncture analgesia
EAP	Electro-acupuncture
FHSL	Front half stride length
f-MRI	Functional-magnetic resonance imaging
HR	Heart rate
HWRL	Hoof withdrawal reflex latency
IA	Intra-articular
LH	Laryngeal hemiplegia
MPJP	Metacarpal-phalangeal joint pathology
MSC	Mesenchymal stem cells
NPV	Negative predictive value
PO	Per os
PPV	Positive predictive value
TCVM	Traditional Chinese veterinary medicine
TPS	Thoracolumbar pain score
TSL	Total stride length

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Traditional Chinese Veterinary Medicine (TCVM) including acupuncture has been used to treat disease and relieve pain in horses for more than 2,000 years.[1-2] As the use of acupuncture has increased over the past few decades in countries where modern Western medicine is the foundation of health care, there has been increasing scientific effort to evaluate this ancient medical modality for objective evidence of efficacy.[3-7] This paper will review the basis of acupuncture and evidence-based clinical application of acupuncture in equine practice including diagnosis and treatment of lameness, pain management, reproductive and other diseases.

ACUPUNCTURE PROCEDURES

The top three acupuncture techniques used currently in veterinary medicine are dry needle (DNAP), aqua-acupuncture (Aqua-AP) and electro-acupuncture (EAP).

Dry Needle Technique

Dry needle (DNAP) is one of the earliest acupuncture techniques. In TCVM it is called "White Needle", *Bai-zhen* (no intentional bleeding). It is the most common acupuncture treatment modality in veterinary and human practice. It involves the insertion of thin sterile needles of certain gauges and lengths depending on species and location of acupoints.

Electro-acupuncture

Electro-acupuncture (EAP) is a growing and common adjunct to DNAP treatments. Historically clinical application of EAP began in China in the 1950's.

It has become common in veterinary practices, especially for analgesic purposes and other pain management.[8-9] Following placement and insertion of the acupuncture needles in appropriate acupoints, the application of a mild electrical current passed through the needles allows a repeatable, more consistent and prolonged therapeutic stimulation. The frequency and amplitude of the electrical current can be adjusted. A high versus low frequency has differing effects on systemic neuromodulation and the amplitude is adjusted to a stimulation threshold tolerated by the patient. There are a variety of EAP unit types available with the ultimate goal of strengthening and altering the needle stimulation. Lower frequency (around 20 Hz) EAP mediates endorphin release and is best for treating pain and muscle spasms. Higher frequency (80-120 Hz) is associated with 5-hydroxytryptamine (5-HT, serotonin) release and may be best to re-educate the motor neurons in paresis and paralysis.[8,10-11]

Aqua-acupuncture

Aqua-acupuncture (Aqua-AP) involves the injection of fluids and soluble products into acupuncture points. Sterile saline, vitamin B12, homeopathic remedies, the patient's own blood, and local anesthetics are most commonly used in Western acupuncture practice. It is used to lengthen and strengthen an acupuncture treatment or used when the patient will not remain calm long enough to keep filiform needles in place.[8] Injection of an animal's own blood has become common for injury, autoimmune and inflammatory disorders.

An interesting example of Aqua-AP is highlighted in a study where eight horses were randomly assigned to four different treatment protocols according to a Latin Square double-blind design in order to explore the clinical sedation effect of Aqua-AP: Group 1) 0.1 ml/kg of saline subcutaneously injected at the cervical region (negative control), Group 2) 0.1 mg/kg acepromazine injected subcutaneously cervical region (positive control, conventional dose), Group 3) 0.01 ml/kg of saline injected into acupoint GV-1 (Aqua-AP, 10% of negative control saline dose) and Group 4) 0.01 mg/kg acepromazine injected into acupoint GV-1 (Aqua AP, 10% dose acepromazine).[12] Signs of sedation were observed in Group 2 (full dose, positive control), Group 3 (Aqua-AP, 10% dose saline) and Group 4 (Aqua-AP, 10% dose acepromazine) at 30 minutes. Only in Group 4 was the sedation effect still present at 60 minutes after the injection. This study indicated that both acupoint injections (at only 10% of negative/positive controls) at GV-1 produced sedation, however, the diluted acepromazine (0.01 mg/kg) injection at GV-1 doubled the length of sedation (60 minutes) at only 10% acepromazine dose when compared to Group 2 full dose acepromazine (positive control).

ACUPUNCTURE POINT SENSITIVITY FOR EQUINE LAMENESS DIAGNOSIS

Digital palpation at key diagnostic acupuncture points for acupoint sensitivity was reported to be used for diagnosis of lameness in horses by American equine practitioners in the 1990's.[13-14] One study reported that out of 327 racing Thoroughbreds examined either for lameness or routine musculoskeletal evaluation, acupuncture point sensitivity diagnosis indicated metacarpophalangeal joint pathology (MPJP) was present in 176/327 (54%) of the affected horses. Of the 176 MPJP horses, 176 (100%) had sensitivity at acupoint LI-18, and 158 (90%) had sensitivity at acupoint SI-16.[15] In this group of horses, 111/176 (63%) were not lame. Of the 65/176 horses that were lame, 18 (27.7%) became sound after intra-articular injection (IA) of mepivcaine hydrochloride in the fetlock. The remaining 47 (72.3%) were lame as a result of extra-articular fetlock or non-fetlock pain.

In a different study, acupoint sensitivity was tested in several different groups of horses showing signs of hoof lameness with conventional diagnosis.[16] Sensitivity at LI-18 was found in 23/30 (77%) horses with chronic heel pain, 31/45 (69%) with acute heel pain and 24/29 (83%) horses with laminitis. Sensitivity at SI-16 was detected in 18/30 (60%) lame horses with chronic heel pain, 27/45 (60%) with acute heel pain and 11/29 (38%) with laminitis. No correlation of foot lameness with acupuncture point sensitivity was found in this study in cases of subsolar abscess, bruised feet, hoof cracks and painful wounds.

A total of 102 client-owned horses were presented for routine acupuncture, reduced performance or lameness.[17] Each horse first underwent a <2-minute screening scan of acupuncture points (AP) and was classified as positive or negative for acupoint sensitivity by the same veterinarian certified in veterinary acupuncture and experienced in performing AP scans and unaware of the presenting complaint in all cases. Each horse was then evaluated for lameness and categorized as lame or sound by a different veterinarian. In the sound group, 40/51 (78.4%) horses had a negative AP scan and 11/51 (21.6%) had a positive AP scan. In the lame group, 9/51 (17.6%) horses had a negative AP scan and 42/51 (82.4%) had a positive AP scan (p<0.001). Acupuncture scanning had a sensitivity of 82.4% to detect lameness and a specificity of 78.4%, with an accuracy of 80.4%. Significant but modest correlations existed between the side of the horse that was positive on the AP scan and the side of lameness. The conclusion of the study was that an AP scan could be a useful, quick screening tool during the physical examination to identify horses that should undergo a full lameness examination and other diagnostic testing.

A Brazilian group performed a soundness exam on 810 athletic horses involved in different disciplines because of poor performance, lameness, refusal to jump, upward fixation of the patella, thoracolumbar spine or sacroiliac pain, suspensory lesions and front hoof problems from 2001 through 2012.[18] They used acupuncture sensitivity on palpation and clinically diagnosed 86 (9.4%) horses with stifle syndrome.

Twenty-two (25.6%) of the 86 horses with stifle syndrome underwent ultrasonography and/or radiography, and lesions were detected in 21 (95.5%) animals. The diagnostic points for stifle syndrome included BL-20, BL-21, local stifle points (e.g. BL-37, 38, 39), GB-27, SP-13, ST-30, and the sacral acupuncture point BL-54. They concluded that acupuncture point sensitivity for diagnosis of stifle disease may be considered a reliable diagnostic method and suggested it be included in routine clinical examination of horses and in reference textbooks as a lameness diagnostic modality.

An equine practitioner from South Africa has reported identifying soft tissue injuries and pain in horses using diagnostic points associated with the Gallbladder Channel with good clinical results.[19-20] Finally, very recently Schmid and Aebischer reported a double-blinded study utilizing 59 client-owned horses randomly chosen and presented for a variety of equine clinical diseases. They evaluated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of scan diagnosis and its correlation with conventional diagnostic methods. In addition, the diagnostic acupoint's reactivity grade was compared to lesion severity to evaluate any correlation between the 2 methods of disease diagnosis. They reported that the acupuncture points scan diagnosis demonstrated a sensitivity of 88.7% (true positive rate), a specificity of 86.9% (true negative rate), NPV value of 98.1% (percentage of non-lesions determined by the scan diagnosis also shown negative in the conventional examination) and positive predictive value of 50.6% (percentage of lesions determined by the scan diagnosis also shown from conventional examination). In addition, a statistically significant correlation (p-value <0.001) between acupuncture point reactivity and lesion severity was demonstrated.[21] These studies demonstrate that acupuncture point diagnosis be an adjunctive tool when diagnosing equine pain and/or structural diseases.

ACUPUNCTURE FOR GENERAL PAIN MANAGEMENT AND MECHANISM OF ACTION

A recent publication reported that acupuncture analgesia mediated through integration of the neuroendocrine-immune network (i.e. neurotransmitters, cytokines, hormones) in the body increased substances such as 5-hydroxytryptamine (5-HT) or serotonin, which increased the pain threshold and improved the absorption of pain inducing inflammatory substances.[22] Another study indicated that low-level laser acupuncture can improve the pain threshold of rabbits, goats, pigs, mice and dogs. It increased the concentration of 5-HT in cerebral spinal fluid which suppresses the evoked cortical potential thereby inhibiting pain.[23] A study using the rabbit knee osteoarthritis model evaluated EAP stimulation (2.0 Hz, continuous wave, 6 volts) of acupoints SP-10, ST-34, ST-35, *Nei-xi-yan*, ST-36 and GB-34 for 30 minutes once per day for 4 weeks. Results showed that EAP reduced the levels of inflammatory factors including IL-1α, TNF-β, and prostaglandin E_2 which alleviates the inflammatory reaction providing notable pain relief.[24]

The effect of different EAP frequencies (2 Hz, 40 Hz, 60 Hz, 100 Hz) on the pain threshold has also been studied using the goat as a model.[25] Two pairs of acupoints *Bai-hui* and *Qi-jia*, *Er-gen* and *San-yang-luo* were stimulated with EAP for 30 minutes. Their results showed that of the frequencies studied, EAP with 60 Hz had the best effect for pain relief. Follow-up studies confirmed that EAP also had an after-effect of pain relief.[25] This study indicated that after being stimulated via EAP with 60 Hz for 30 minutes, the pain threshold of goats increased and reached its peak at Hour 0 (i.e. at the end of EAP stimulation). The pain tolerance then decreased gradually to the baseline by Hour 5, however, by Hour 6 the pain threshold began to rebound and reached a second peak at Hour 8, and then gradually fell again reaching baseline at Hour 12. The mean pain threshold during the time from 0 to 12 hours after EAP was higher (p<0.05) than that at 0.5 hour before EAP, which showed that the EAP-induced analgesic after-effect lasted for at least 12 hours in goats.[26]

The efficacy of various frequencies of EAP for pain relief in 22 horses was systematically reviewed in a clinical trial.[27] Focused radiant light/heat was used as a noxious stimulus and was directed onto the equine pastern to elicit the classic flexion-withdrawal reflex. Hoof withdrawal reflex latency (HWRL) was defined as the time (in seconds) between lamp illumination and the withdrawal of the hoof. The results indicated that the HWRL is a valid measurement to assess pain perception and document pain relief from acupuncture. The study also demonstrated that EAP treatments for 30 minutes at high frequencies (120 Hz) induce a stronger analgesic effect than 30 minute EAP treatments at low frequencies (20 Hz) in local regions. The EAP treatments at lower frequencies, however, induced a longer analgesic effect.

A double 3x3 Latin Square design was applied in studying experimental lameness in 6 horses. Lameness was produced in each subject by tightening a setscrew against the sole of the hoof.[28] Lameness grading scores of 0, 1, 2 and 3 were used to evaluate the severity of lameness. Three types of stride length were measured: total stride length (TSL), front half stride length (FHSL) and back half stride length (BHSL). The difference between FHSL and BHSL was defined as DFB (difference front/back). The DFB increased significantly when the horse was lame suggesting that the DFB could be used as an objective parameter to measure lameness in horses. Plasma concentrations of β-endorphin, adrenocorticotropic hormone (ACTH) and cortisol were also measured in both experiments. The use of EAP was significant for: increased HWRL and reduced lameness score, while simultaneously increasing the plasma β-endorphin concentration. These results indicate that the release of β-endorphin may be the pathway for acupuncture pain relief in this experimental setting. None of the acupuncture treatments altered the ACTH

concentrations which indicates that ACTH is not involved in EAP analgesia.

A different result was reported, however, in a pilot study which indicated that acupuncture treatment had no effect on pain in horses.[29] Nine horses with palmar heel pain which varied from 1 to 3 on a lameness scale were randomly assigned to an acupuncture or control group. Twice weekly visits on non-successive days were made to each horse. Horses in the treatment group received 20 min of DNAP and EAP at each visit while horses in the control group received no treatment. The same acupuncture points were applied to each horse in the treatment group (n=5): *Bai-hui*, BL-11, BL-13, PC-1, HT-9, LU-1, LU-11 with dry needle; and SI-9 and LI-11 bilaterally with EAP at 2 to 5 Hz. The researchers found with observational grading that all 4 horses in the control group maintained the same grade of lameness through the duration of the study or improved on 1 or both limbs by no more than 1 grade of lameness. Of the 5 horses in the treatment group, 3 showed improvement of 1 lameness grade on 1 or both limbs, 1 horse did not change, and 1 horse's lameness worsened through the course of the study. There was no statistically significant difference in grade of lameness between treatment and control animals at both initial and final assessment. The researchers in this study concluded that acupuncture did not reliably modulate palmar heel pain in horses.[28] A 2006 systematic review indicated that there was no compelling evidence to recommend or reject acupuncture for any condition in domestic animals including horses and dogs even though some encouraging data did exist that warranted further investigation in independent rigorous trials.[30]

Different outcomes of acupuncture on lameness may be associated with the fact that lameness itself can be subjective to study. Objective gait analyses using inertial sensors were adopted in a blinded and crossover study in mildly lame horses. Objective gait analyses using quantitative sensor based gait analysis were performed before and after each treatment and at 1, 3 and 7 days after the last treatment.[31] Horses were assessed at the trot in a straight line on a hard surface (condition 1) and on the lunge on the left (condition 2) and right reins on a soft surface (condition 3). Acupuncture treatment was found to decrease hip hike difference under all assessment conditions including condition 1: control, 6.3 ±6.4 mm versus treatment, 0.2 ± 6.4 mm (p = 0.007); condition 2: control, 9.7 ± 7.8 mm versus treatment, 2.8 ± 7.8 mm (p = 0.032); condition 3: control, 7.3 ± 6.3 mm versus treatment, 2.7 ± 6.4 mm (p = 0.003)]. This study indicated that acupuncture treatment changed the horses' gaits (appreciable by objective analyses), with treated horses moving in a more symmetrical manner, which suggests a lesser degree of discomfort.

ELECTRO-ACUPUNCTURE ANALGESIA FOR SURGICAL PROCEDURES

Electro-acupuncture analgesia (EAA) was used for surgical procedures without anesthesia drugs for the first time in 1958.[32-33] In a different study, an EAA protocol was used for surgery on horses and donkeys.[34] The procedure was initiated by using a 20 Hz frequency for 10 minutes and then gradually increased to 55 Hz for another 10-20 minutes which was sustained for the entire surgical procedure. Surgeries on the head and neck, chest wall, thigh and abdomen, as well as castration were performed on 18 healthy experimental animals which included 10 stallions and 8 mares, 8-15 years old, weighing 350 to 450 kg. In addition, multiple surgical protocols (suturing skin lacerations, subcutaneous mass excision, hernia repair, castration) were successfully performed using the EAA protocol without drug induced general anesthesia in 7 clinical cases which included 2 foals, 1 gelding, 2 stallions and 2 donkeys. Each animal, whether clinical or experimental, had an independent acupoints plan, depending on the site of surgery with electro-acupuncture stimulation throughout the duration of the surgery.[34]

In another report, twenty-three surgeries in 23 cattle described the effectiveness of EAP induced surgical anesthesia/analgesia relative to regional needle placement.[35] The locations of regional EAP were divided into 4 groups: a dorsal acupoint group [*Tian-ping* (GV-5) and *Bai-hui*, (n= 7)]; a lumbar acupoint group [*Yao-pang* 1 (BL-21), *Yao-pang* 2 (BL-23), *Yao-pang* 3 (BL-24) and *Yao-pang* 4 (BL-25), (n=5)]; a combined dorsal-lumbar acupoint group (n=8); and a control group using the last intercostal space to the femoral area as sham acupuncture points (n=3). Surgeries performed on cattle in the dorsal acupoint group and assessed for degree of analgesia were 2 laparotomies, 3 umbilical hernia repairs and 2 castrations. Similarly, surgeries performed on cattle in the lumbar group were 5 omentopexy surgeries for correction of left-sided displacement of the abomasum, whereas surgeries performed on the dorsal-lumbar acupoint group consisted of 4 omentopexies for correction of left-sided displacement of the abomasum, 1 omentopexy for correction of right-sided displacement of the abomasum, 2 rumenotomies and 1 cesarean section. The acupoints were stimulated with currents of 2-6 V (30 Hz) in dorsal acupoint group, 0.5-2.0 V (30 Hz) in lumbar acupoint group and 0.3-2.5 V (30 Hz) in dorsal-lumbar acupoint group. The results of their analyses showed that the recumbency and induction time in the dorsal acupoint group approximated 10 seconds to 1 minute respectively and the induction time of analgesia was 1-6 minutes, in all animals except 1 who failed to respond to the EAP. The induction time of analgesia in the lumbar and dorsal-lumbar acupoint groups was slightly longer at approximately 10 minutes. The authors concluded that the use of their dorsal acupoint protocol in responsive animals might be useful in providing analgesia for surgeries requiring the patient to be in a recumbent position whereas the use of the lumbar and dorsal-lumbar acupoint protocol might be useful for standing surgeries in cattle.[35]

A clinical trial (cross-over design) was conducted to compare the effects of EAP and butorphanol on rectal analgesia in mares (n=8) using controlled rectal distention as a noxious stimulus.[36] Animals were also monitored for

changes in hemodynamic and respiratory variables. Each horse received saline (0.9% NaCl) solution (0.01 mL/kg, IV; control treatment), butorphanol tartrate (0.1 mg/kg, IV), or 2 hours of EAP at acupoints *Bai-hui*, bilateral BL-21, 25, 27 and ST-36 (right side only). The order of treatments in each mare was randomized. At least 7 days elapsed between treatments. A balloon was inserted in the rectum of each mare, and controlled distention of the balloon (pressures of ≤ 220 mm Hg) was used to measure nociceptive rectal pain threshold. Both butorphanol and EAP provided statistically equal analgesia to induced rectal stimuli (mean +/- SD, 214 +/- 24 vs 174 +/- 35 mm Hg of balloon pressure, respectively). The conclusions and clinical relevance were that EAP and butorphanol (0.1 mg/kg, IV) may provide useful rectal analgesia in horses and EAP produces less effect on hemodynamic and respiratory variables when compared to butorphanol.

ACUPUNCTURE FOR TREATMENT OF OTHER CONDITIONS IN HORSES

Back Pain

A prospective study was conducted to evaluate the use of EAP in the treatment of horses with signs of chronic thoracolumbar pain.[37] Fifteen horses were randomly allocated to 1 of 3 treatment groups. Horses in Group 1 received EAP stimulation (once every 3 days for 5 treatments), those in Group 2 received phenylbutazone (2.2 mg/kg [1 mg/lb], PO (per os), q (every) 12 hours, for 15 days), and those in Group 3 received 0.9% NaCl saline solution (20 mL, PO, q 12 hours for 15 days). Thoracolumbar pain scores (TPS) were evaluated before (baseline) and after each treatment. The TPS in horses receiving phenylbutazone and saline solution did not change significantly during the study (p=0.999 and p=0.535 respectively). After the third treatment, TPS in horses receiving EAP stimulation were significantly lower than baseline (p<0.01) and decreased from 6.0 ± 0.6 to 2.1 ± 0.6. The statistically significant lower scores were maintained through follow-up 14 days after the 5th treatment. These results provided evidence that three sessions of EAP treatment can successfully relieve signs of thoracolumbar pain in horses and the analgesic effect induced by EAP can last for at least 20 days. Alternatively, the oral administration of phenylbutazone was not found to effectively relieve signs of thoracolumbar pain.

Another clinical trial found that EAP relieved chronic back pain in performance horses.[38] This study was a randomized, double blind, controlled trial to evaluate EAP as a treatment for back pain in sport horses. Objective measurements of pain threshold levels were obtained with a pressure algometer. Twenty-three horses with chronic back pain were divided into control (n=7) and treatment (n=16) groups. Trigger (painful) points were identified on each horse and baseline pain threshold measurements were taken. The control group received sham EAP treatments with no needle penetration or electrical stimulation. Routine EAP was performed in the treatment group using filiform acupuncture needles inserted into GV-20, GV-6 and bilaterally at BL-26, BL-54, BL-21 and BL-17. Needles were connected to 5 pairs of electrical wires and an electrical impulse (4.5 volts) was delivered at a frequency of 20 Hz for 15 minutes and 80-120 Hz for 15 minutes. Both sham and control EAP treatments were given over the course of 5 sessions, each spaced 3 days apart and all horses were rested during the study period. After 5 treatments, pressure induced pain was statistically significantly reduced at the trigger points in the treatment group when compared to the control group using an unpaired t-test (p=0.034). The conclusion was that EAP and rest is an effective treatment for sport horses with chronic back pain and is better than sham EAP and rest over a 15-day period. Similar findings have also been reported by other researchers.[39-40]

Laminitis and Navicular Disease

A study was conducted to compare lameness levels before and after acupuncture treatments in horses with chronic laminitis.[41] Twelve adult horses with chronic laminitis received 2 acupuncture treatments 1 week apart. The points were treated using dry needle, hemo-acupuncture and aqua-acupuncture. Lameness level was objectively evaluated using a commercial inertial sensor-based lameness evaluation system[a], as well as routine examinations following the American Association of Equine Practitioners scoring before the first and 1 week after the second acupuncture treatment. Data were analyzed using Wilcoxon signed-rank test and p-values < 0.05 were considered statistically significant. Both the inertial sensor-based lameness evaluation system (p = 0.0269) and routine lameness examination (p = 0.0039) showed a significant reduction in lameness severity. This clinical trial supports using acupuncture, along with other treatment options, in treating chronic equine laminitis.[41] Researchers in a different clinical trial, however, obtained conflicting results which indicated that EAP used for treatment of chronic laminitis (n = 5) or navicular disease (n = 5) did not have a significant difference when comparing clinical scores between experimental and control groups.[42] The authors of this study pointed out that the small number of animals per group may have obscured a positive treatment effect with acupuncture. In addition, the lower frequency used in this study (5 Hz for 20 minutes, local acupoints) may be a factor as it countered the relatively recent study in which a high frequency (120 Hz for 30 minutes, local acupoints) induced a stronger analgesic effect than low frequencies (20 Hz for 30 minutes) in the local foot region.[42] Other reports support the use of acupuncture for the treatment of laminitis and navicular syndrome.[43-44]

Cervical Stiffness

Eighteen (18) horses diagnosed with cervical stiffness were randomly divided into a Test Group and a Control Group.[45] Horses in the Test Group received 3

acupuncture treatments (DNAP and EAP), 7-10 days apart, using bilateral 1-2 inch needles dependent on anatomic site: DNAP at *Shen-shu*, BL-62, SI-3, LIV-3, LI-4 and EAP (20 Hz, 10 minutes) at BL-10 + *Jing-jia-ji* (C3-4), *Jing-jia-ji* (C4-5) + *Jing-jia-ji* (C6-7). Horses in the Control Group received treatment on the same schedule but using 0.5mm press needles at non-acupuncture points. From each horse, two measurements of cervical lateral bend were taken prior to the first treatment and again 1 day after the last treatment. One measurement was the amount of bend before refusal (maximal bend, R1) and the other was amount of bend before compensation (Pre-compensation, R2). The comparison of the changes between the Test Group (9.83 ± 8.87) and the Control Group (-6.83 ± 15.26), based on the Wilcoxon Rank Sum test, revealed that the mean R1 change in the Test Group was significantly larger than that in the Control Group (p = 0.019). The same analysis on R2 bend measurement reached the same conclusion (12.22 ± 8.82 test vs. -5.17 ± 13.07 control; p = 0.008). This study demonstrated that acupuncture can improve lateral bend in horses and can be an effective treatment for cervical stiffness.

In a retrospective study, case files for nineteen animals (13 dogs and 6 horses) with wobbler syndrome ranging in age from 4 months old to 14 years old were reviewed.[46] Dog breeds included: 4 Doberman Pinschers, 2 German Shepherds, 2 Great Danes, 1 Greyhound, 1 Rottweiler, 1 Weimaraner, 1 Dalmatian and 1 Australian cattle dog. Horse breeds included 2 Thoroughbreds, 1 Standardbred, 1 Warmblood, 1 Andalusian, and 1 Saddlebred. Six dogs and 1 horse presented with at least one conventional medical diagnostic test, including radiographs, myelogram and magnetic resonance imaging (MRI). Twelve animals (7 dogs and 5 horses) presented with clinical signs typical of wobbler's syndrome, including ataxia and/or hind end weakness. Assessment of clinical signs of the animals varied based on degree of neurological dysfunction or pain measured on a 5 point grade scale. All 19 cases were treated with both acupuncture and Chinese herbal medication. Acupuncture treatment included dry needle at *Bai-hui* and BL-23; electro-acupuncture (20 Hz for 5 to 10 minutes + 80-120 Hz for 15 to 20 minutes) at up to seven pairs of acupoints: GB-20 + GB-21 (crossed right to left and left to right), local *Jing-jia-ji* points for affected cervical vertebrae, ST-36 + GB-34, BL-54 + KID-1, GV-14 + GV-20 in dogs, or BL-10 + BL-11 in horses; Aqua-AP (vitamin B12) at *Jing-jia-ji*, BL-62, SI-3, KID-6. One session of acupuncture treatment per 1 to 4 weeks was given for a total of up to 6 sessions. The Chinese herbal medicine, Cervical Formula[b] (*Jing Tong Fang*) was used orally for all patients. Double P II [b] (modified *Da Huo Luo Dan*) was given orally to patients that had a neurological grade of 2 or higher. Body Sore [b] (*Shen Tong Fang*) was given orally to patients with neck pain and used as needed. After 5 to 8 sessions of acupuncture treatments and 2 to 3 months of herbal medication, 10 (52.6%) out of the 19 cases had complete clinical recovery, and 8 (42.1%) had a substantial improvement (improved at least one neurological grade). Only 1 of 19 cases (5.3%) had no improvement. All 18 cases that responded to TCVM were followed for at least 6 months, and demonstrated stable clinical signs and good life quality (including daily walk, normal urination/defecation, good appetite).

Reproductive Disorders and Mastitis

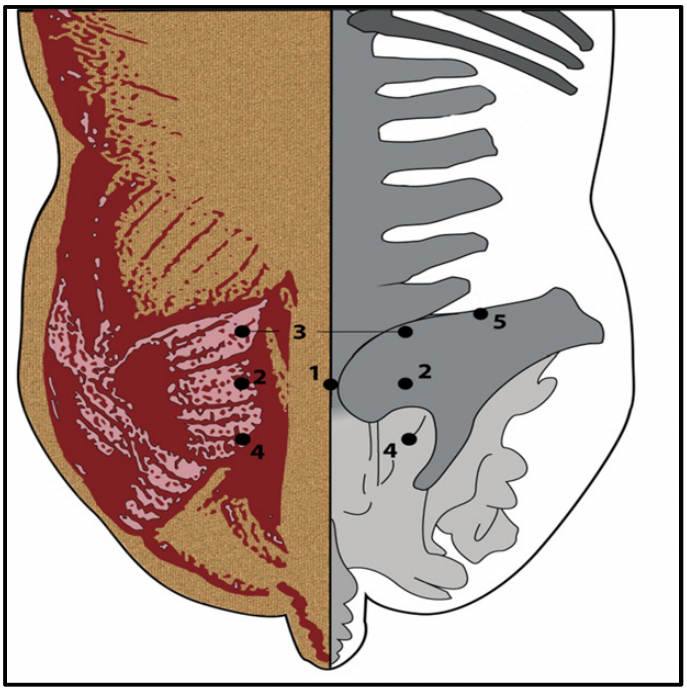
The treatment of reproductive disorders and the promotion of fertility represent cornerstones of the equine and bovine industries. Acupuncture has anecdotally produced excellent results for treating mares with uterine fluid and/or urine pooling, especially older, pluriparous mares.[47] In a bovine study, 57 dairy cows that were diagnosed with infertility due to inactive ovaries were randomly assigned into four groups: electro-acupuncture (n=15), Aqua-AP (n=15), hormones (n=15) and control (n=12). Four acupoints used in both EAP and Aqua-AP groups were *Bai-hui* and GV-1, and bilateral *Yan-chi*. In the EAP group an alternating frequency setting between 80-100 Hz was used for 30 minutes once a day for three consecutive days.[48] For Aqua-AP, 15 ml of 5% dextrose was injected into each of these 4 points, once daily for 3 consecutive days. For the hormone group, FSH (100-200 units per injection) was given intramuscularly twice, 48 hours apart. For the Control Group, no treatment was given. After treatment in the EAP group, 13 out of 15 dairy cows (86.7%) had a normal estrus, were inseminated, and 12 (80%) were diagnosed pregnant. In the Aqua-AP group, 9 out of 15 cows (60%) had a normal estrus, were inseminated, and 7 (46.7%) were diagnosed pregnant. In the hormone group, 12 out of 15 (80%) had a normal estrus, were inseminated, and 11 (73.3%) were diagnosed pregnant. In the control group, 4 out of 12 cows (33.3%) had a normal estrus, were inseminated, and 2 (16.7%) conceived. The estrus and pregnant rates were not significantly different between the EAP and hormone groups, and between the Aqua-AP and control groups, but rates in both EAP and hormone groups were significantly higher than the control and Aqua-AP groups. In both EAP and hormone groups, the milk progesterone level increased significantly after the treatment. This study indicated that EAP was an effective therapy for infertility due to inactive ovaries.

A clinical study demonstrated that Aqua-AP of herbals at GV-1 can prevent retained placenta in cows.[49] One hundred and twenty four pregnant dairy cows were selected to be in an untreated control group and observed after calving to determine the retained placenta rate for the farm. Fifty-two pregnant dairy cows from the same farm were selected for the study and randomly assigned to two groups: 30 cows in the herbal *Dang Hong Fu* group and 22 cows in a saline control group.[49] Immediately after calving 40 ml of *Dang Hong Fu* (40 grams of dried herbs) were injected into GV-1 in the herbal group and 40 ml of physiological saline were injected at the same site in the saline control group. Both groups were observed for retained placentas and the time until placental expulsion was recorded in the others. The retained placenta rate for

untreated cows that received no treatment was 35.5% (44/124). The incidence of retained placenta in the *Dang Hong Fu* group was 16.7% and in the saline control group 31%. The time for expulsion of placental membranes was a mean of 9 hours (range 3.5-24 hours) in the *Dang Hong Fu* group and a mean of 14.7 hours (range 3.0-24 hours) in the saline control group. When compared to the untreated control group, *Dang Hong Fu* aqua-AP at GV-1 significantly reduced the incidence of retained placentas (p=0.047; <0.05), but saline aqua-AP did not (p=0.740; >0.05). Herbal aqua-AP may offer an easy treatment method to reduce the incidence of retained placenta in the cow with no observed adverse side effects.

Promising human studies have prompted calls for mergers between Chinese and conventional approaches.[50] The authors of a systematic review involving a total of 12 clinical trials and 2,177 patients, concluded that the effect of acupuncture on human male infertility was equally effective as prescribing herbals within traditional Chinese medicine (TCM), and its effectiveness is enhanced when applied in combination with either TCM or conventional medicine.[51] In another study, 114 human patients of *in vitro* fertilization embryo transfer (IVF-ET) treated with a standard long-term program at luteal phase were randomized into an acupuncture group or a control group, 57 cases in each.[52] In the acupuncture group, at the beginning of ovulatory induction, moxibustion was applied to CV-8, and acupuncture at CV-3, CV-4, CV-6, SP-6, SP-9, SP-10, ST-36, PC-6, LI-4 and LIV-3 periodically from oocyte aspiration until the time of embryo transfer (i.e., the time the embryo was transferred to the patients) for one session of treatment. There was a total of 3 treatment sessions in the study. Results indicated that acupuncture and moxibustion affect estrogen level on hCG day, improve high-quality embryo rate, endometrial blood flow state and morphology so that the endometrial receptivity is increased.[52]

Equine studies, however, have had conflicting results.[53-55] In addition to the fact that human oocytes are capable of *in vitro* fertilization whereas equine oocytes are not and require sperm injection, one reason for the variable results is likely related to the lack of the important classical equine acupoint, *Yan-chi*, that is a specific acupoint for equine infertility (Figure 1 and Table 1).[56] Secondly, the practitioner must rely on the classic Chinese differentiations of specific patterns for point recommendations. For example, ovulatory dysfunction, regardless of cause, is viewed as one of a number of possible deficiency patterns with treatment aimed at strengthening the deficient area of the body. Improved clinical results may include using acupoints SP-6, KID-3, KID-7 and KID-10. And third, CV-4 and CV-6 in particular are among a group of points found to possess endocrine effects pertinent to reproduction, specifically ovulation in humans. The use of these acupoints in the mare, while clinically efficacious, are difficult as well as dangerous to access, thus it is almost impossible to treat these acupoints. Other reproduction related acupoints may be used such as *Yan-chi*, *Shen-shu*, *Shen-peng* and *Shen-jiao* if the diagnoses indicate (Figure 1 and Table 1).



1=*Bai-hui*, 2=*Shen-shu*, 3= *Shen-peng*, 4= *Shen-jiao*, 5= *Yan-chi*

Figure 1: Acupuncture points used commonly for infertility in horses.[56]

Table 1: The commonly used classical acupoints in horses with anatomical location and indication for use.[56]

Acupoint	Anatomy*	Indication
Bai-hui	On dorsal mid-line at the lumbosacral space	Lumbar pain, hindquarter pain or weakness, general calming, *Yang* Deficiency, infertility
Shen-shu	2 *cun* lateral to *Bai-hui*	Lumbar pain, infertility, general pain management, hindquarter weakness
Shen-peng	2 *cun* cranial to *Shen-shu*	Same as *Shen-shu*
Shen-jiao	2 *cun* caudal to *Shen-shu*	Same as *Shen-shu*
Yan-chi	Midpoint between top of tuber coxa and *Shen-peng*	Female or male infertility, poor athletic performance, hindquarter pain/arthritis

**cun* is an acupuncture point measurement unit. The length of the first tail vertebra or the width of the last rib is equal to 1 *cun.*

Stress Response

Acupuncture has been shown to have the beneficial effect of reducing stress responses in horses.[57] A study was conducted to compare the effects of injecting the standard dose of acepromazine (0.1 mg/kg, IM) to Aqua-AP (1/10 of the standard acepromazine dose at the acupoint GV-1) on the stress responses of healthy horses undergoing road transport for 2.5 hours. Four different treatments were applied immediately before loading, with 8 animals/treatment: injection of saline or acepromazine (0.1 mg/kg, IM) at the base of the neck and injection of saline or 1/10 acepromazine (0.01 mg/kg) at the GV-1 acupoint. The road transport increased heart rate (HR), respiratory rate, body temperature, and serum cortisol of the untreated horses (injected with saline at the base of the neck). Aqua-AP (0.01 mg/kg acepromazine) at GV-1 reduced the average HR and transport-induced increase in HR at unloading, without changing the other variables. On the other hand, acepromazine at conventional dose (0.1 mg/kg) produced significant sedation and reduced the transport-induced increase in respiratory rate but without preventing the stress-induced increase of cortisol.[57] Similarly, sedation was induced in horses receiving a 20-minute session of dry needle acupuncture at GV-1, HT-7, GV-20 and BL-52 assessed by the failure to fully respond to the sudden appearance of a multi-colored umbrella as a stressor.[58]

Emergency Resuscitation

Acupoint GV-26, which in the horse is located just below the nostrils at the midpoint of the philtrum nasale, can be used in an emergency situation to resuscitate animals including the horse (Figure 2).[59] A study of 69 cats and dogs reported that acupuncture at the acupoint GV-26 restored respiration to normal or near normal rates within 10 to 30 seconds of needle insertion in 100% of animals if there was no concurrent cardiac arrest. When cardiac arrest occurred and vital signs were absent the revival rate was 43%.[60] In a clinical report with patients following narcotic induced narcosis, 243 cases in 17 different species of domestic, exotic animals and birds; acupuncture resuscitation approached the 100% efficacy reported in clinically healthy dogs.[61] In animals affected with different diseases, however, the success of intervention was smaller (77.47%). In zoo animals suffering from narcosis, the resuscitation effectiveness achieved was 92.6%. The resuscitation effect was based not only on strictly determined acupoints but also on diffusive irritation of the respective point and its surroundings by acupressure. These studies indicate what has also been widely discussed and anecdotally demonstrated by practitioners in the field for many years.

Metabolic Capacity

Eighteen Thoroughbred racehorses were randomly divided into three groups (n = 6/group): negative control, sham aquapuncture (positive control) or treatment aquapuncture groups. Horses in the treatment group received 5 mL distilled water injected into acupoints including *Bai-hui*, ST-30, ST-36, GB-27, SP-13 and BL-22 along with hemo-acupuncture (bleeding) at TH-1 and BL-67. The horses completed six Aqua-AP sessions during a 3-week study period (twice weekly). All horses had the same trainer/jockey and completed at least 8 months of training and racing. The horses did not race during a 2-week period before and after the experiment. Cardiovascular values were not different between times or groups. The metabolic capacity values V_{La4} and V_{200} were statistically greater after treatment only for the acupuncture group compared with pretreatment. Creatine kinase concentrations were statistically greater after exercise for all groups, and aspartate aminotransferase

concentrations were not different between times or groups. This study indicated that acupuncture increased the anaerobic metabolism of Thoroughbred horses without interference in cardiovascular performance or release of muscle enzymes in medium load exercises.[62]

Laryngeal Hemiplegia

Laryngeal hemiplegia (LH) is an important disease related to poor performance and upper respiratory noise in horses.[63] While surgical procedures may be effective in young horses with grade III or IV disease, surgical procedures may be inconvenient for the treatment of LH in horses if the problems occur during the sale seasons or may be rejected as a treatment by some horse owners. Owners may therefore try to find alternative methods for the treatment of recurrent laryngeal neuropathy. Thoroughbred horses (n=18) referred to the acupuncture service at the Veterinary Medical Center at the University of Florida for the treatment of LH were involved in this study. All horses underwent endoscopy with left-sided flaccid laryngeal tissue that adducted during breathing noted during the exam. The hemiplegia endoscopic grades ranged from IIa to IIIb. Electroacupuncture was performed once per week for a total of 3 to 7 times depending on the severity of hemiplegia. The acupoints used were LI-15, LI-17, LI-18, GB-21, CV-23, ST-9, SI-17, *Hou-bi* and *Hou-shu*. The EAP treatment used 20 Hz for 10 minutes, then at 80 to 120 Hz for 10 minutes. All horses had endoscopic examinations by independent (blinded) equine practitioners after 1 or 2 days following the last EAP treatment. The endoscopic grades of hemiplegia had improved in all horses, to between normal and grade IIb (minor flutter or delay in laryngeal movement). The respiratory noise during training also appeared to be improved after the treatment.[64]

Figure 2: The GV-26 acupuncture point is commonly used for emergency resuscitation in horses. The acupoint is located on the midline, on the upper lip, between the ventral limits of the nostrils.[59]

Stem Cell

The effects of EAP on the mobilization of stem cells in horses, mice, rats and humans has recently been studied.[65] In all four species, equivalent acupoints LI-4, LI-11, GV-14 and *Bai-hui* (or GV-20 in humans) were used with EAP stimulation of 30 Hz for 45 minutes. Stimulation using EAP in humans, horses, mice and rats resulted in mobilization of mesenchymal stem cell (MSC)-like cells into the systemic circulation. When examined by an *in vivo* angiogenesis assay the MSC origin of EAP-mobilized cells was supported because the cells did not directly form blood vessels or lumenize, thereby supporting a nonendothelial origin. Mobilization of MSC-like cells was preceded by a time-dependent increase in plasma norepinephrine levels and was blocked by pretreatment with propranolol. Analysis by fMRI in EAP-stimulated rats revealed increased functional connectivity between the anterior hypothalamus and the amygdala. Pharmacological disinhibition of these regions enhanced sympathetic activation and similarly resulted in release of MSC-like cells into the circulation. Following partial rupture of the Achilles tendon, EAP produced long-lasting and powerful analgesia and generation of increased type 1 collagen content, indicative of tendon injury remodeling; however, this effect was blocked in propranolol-treated rats. Thus, EAP activates the sympathetic nervous system to mobilize MSC-like cells into circulation which can be used to enhance tissue repair and provide analgesic relief.

CONCLUSION

Acupuncture stimulation, especially electro-acupuncture, can release neurotransmitters such as 5-HT, serotonin and endogenous opioids including β-endorphin, which appear to be the main pathways in which acupuncture relieves pain. Although the strength of the clinical trials cited above vary, acupuncture has been shown to be a viable integrative treatment for back pain, foot pain, cervical stiffness, laryngeal hemiplegia and infertility in horses. Acupoint sensitivity on palpation may be useful for the assessment of lameness along with conventional diagnostics in horses. Future well-designed studies are needed in order to strengthen the recommendation for acupuncture in the diagnosis and treatment of clinical conditions in horses.

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Declaration of Ethics: Authors declare that they have adhered to the Principles of Veterinary Medical Ethics of

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FOOTNOTES

a. Lameness Locator™, Equinosis LLC, Columbia, MO USA

b. Jing Tang Herbal, Ocala, FL USA

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