Observational Study Monitoring Pain in 25 Dogs Comparing Novel Non-invasive Device and Veterinary Diagnosis to Differentiate Location and Magnitude of Pain
Introduction
Currently, persistent pain is principally measured by self-report in humans or validated pain scales in veterinary medicine based on physical exam and activity. There has been an increase in the number of pain-related neuroimaging studies and complementary human experimental techniques including quantitative sensory testing but a direct objective biosignal has not yet been elucidated. In this study, a pain monitor utilizing a direct biosignal, employing skin-mounted sensors, was evaluated and compared to veterinary diagnosis. The use of a novel biosignal holds promise for quantitative correlation to both animal and human pain diagnosis.

Goals
Animals cannot verbalize their pain levels directly. The following data analyzes veterinary diagnosis compared to PainTrace® biosignal to establish statistical correlation and demonstrate potential utility as a pain measure.

For illustration purposes, we show the correlation of self-reported VAS (Visual Analog Scale), commonly employed in human studies in comparison to a biosignal (Figure 1). VAS Self-report is graphed in blue and PainTrace® (PT) values are graphed in red.

- More Pain Equals a More Negative Number
- A Positive Number Equates to No Pain (or increasing levels of wellness)

![Figure 1. Self-reported pain (blue) vs. PainTrace® biosignal (red) in axillary nerve injury](image)

During this visit pain was produced by scratching the area of skin associated with the Sergeant’s Patch at 45, 200, and 290 seconds. Pain levels recorded with the BioTraceIT™ device closely correlate with self-report of pain. Note: The VAS equal to 6 at 45 seconds was not recorded and was added for graphing purposes. All recorded VAS are denoted by the yellow diamond markers.

Materials and Methods
In an observational study involving 25 dogs, pain was monitored using the skin mounted sensors that acquire a novel biosignal during veterinary exams. Dogs were selected when scheduled as rehabilitation referrals, or pain exam/recheck appointments. Three dogs represented annual health exams. All dogs were submitted to a thorough exam evaluating the entire anatomy for comparison of painful and non-painful diagnosis. Pain diagnosis was separated into 5 categories: pain, discomfort, spasms, tightness, and trigger points (see below for definitions). To compare the pain diagnosis for correlation between veterinary and medical device pain measurement an unpaired T test was employed.

The PainTrace® wearable monitor from BioTraceIT™ (Figure 2) was employed to measure and process pain signals capturing a biosignal much like an ECG. The device utilizes a set of non-invasive, simple, fast application disposable sensors applied directly to the skin. A small 25mm area was shaved and cleaned via alcohol prep prior to application of the sensors to the skin. A one-minute calibration period was employed prior to commencing veterinary analysis which comprised – anatomical exam, recorded examinations notes, and associated diagnostic observations. The PainTrace®, Bluetooth® enabled device, captured biosignal data for subsequent analysis.

![Figure 2. Wearable PainTrace® device](image)

Case Example
Figures 3(A) and 3(B) represent PainTrace® profiles during patient exams. The exam is focused on the injured left forelimb. On June 2, 2016, a 6 y.o., 33 kg, greyhound presented with inflamed digit of left forelimb was found to be non-responsive to the veterinary exam, and appeared to be masking pain. PainTrace® was utilized and it became evident pain was experienced upon weight bearing on the affected left foot. On June 2, 2016, a 6 y.o., 33 kg, greyhound presented with inflamed digit of left forelimb was found to be non-responsive to the veterinary exam, and appeared to be masking pain. PainTrace® was utilized and it became evident pain was experienced upon weight bearing on the affected left foot.

The following describes the BioTraceIT™ data in Figure 3(A) below and the associated exam from left to right: 1) Minor discomfort during right forelimb exam, 2) Pain (negative deflection) during left forelimb exam, 3) Noted negative deflection when dog weight bearing on left fore-

![Figure 3(A). Left forelimb pain during veterinary examination of 5th digit non-union](image)
The greyhound’s inflamed 5th digit was diagnosed as a fracture non-union, which is defined by an arrest in the fracture repair process with progressive evidence of non-healing most commonly related to inadequate fracture stabilization and poor blood supply. Subsequently, an amputation was completed on October 5th.

On October 11, 2016, the dog returned for a follow-up visit five (5) days post-amputation 5th digit with fracture non-union in affected left forelimb. Pain was managed with 300 mg (~10 mg/kg) TID, 30 mg Codeine (~1 mg/kg) q4h, and Metacam (~0.1 mg/kg). From the PainTrace® signal, the post-op follow-up appears to represent managed acute pain based on the absence of any significant negative PainTrace® signal deflections. The overall PainTrace® baseline is more negative potentially denoting an increased overall, or chronic, pain level. Veterinary exam also confirmed the absence of acute pain.

Results

Based on veterinarian diagnosis, events were categorized into pain (N=89) and non-pain (N=151) groups. Pain, as defined in this study, was a reaction to manipulation or movement that includes vocalization, and/ or strong reactions of withdrawal or aggression. This may include rapid “dropping” of hind end with spinal manipulation, turning to look at the examiner or moving away, increases in anxious behavior, avoidance and either panting or holding of breath. Using an unpaired t-test, a significant difference between the population means was noted (p<0.001).

Since pain vs. non-pain states are clearly differentiated, we wanted to determine if discrete “levels” of pain could be differentiated. As a result, the following pain-related classifications were appended (Table 1). Discomfort (N=8) – less intense signs of pain such as mild vocalization, pulling limb up with palpation but not trying to avoid, less intense physical reactions (i.e. mild dropping of head with neck pressure). Spasms (N=34) – Palpable or visible fasciculations or twitches in muscles when palpated either at the site of palpation or nearby. Spasms may also occur with physical movement involving the affected area. Tightness (N=10) – Increased tone and firmness in a muscle or region of a muscle with or without trigger points that causes reduced range of motion or increased resistance to range of motion. Trigger points (N=6) – Pain on specific regions of a muscle which usually also includes a firm nodule or region in the tissue.

The five (5) groups classified as: pain, discomfort, spasm, tightness and trigger points, were compared pairwise against each other using Kruskal-Wallis test (Table 2) to check for significant difference amongst each group in a pairwise manner, see Table 2.

Discussion

In this observational study, we have established that the novel pain monitoring device, PainTrace®, quantifies pain biosignals correlating with veterinary diagnosis with p values < 0.001. Statistically significant differentiation between pain and discomfort versus additional observations related to spasms, tightness, and trigger points can also be evaluated. The utility of the device, as a wearable for extended periods of data collection and multi-activity pain monitoring — e.g. exam, rehabilitation, and pre/post-surgery, supports long term follow-up care to determine the effectiveness of treatment(s). Additional human studies in both the rotator cuff and lower back have been completed to demonstrate the versatility of the device in other species, as well as orthopedic protocols in both canine and equine patients. Further animal studies are in progress and warranted based on outcomes in IRB approved human studies employing PainTrace®. PainTrace® values compared to human self-reported pain have produced p values < 0.001. Studies with less than twenty participants can achieve a power of 80%.

Based on these positive outcomes, we plan to further the potential of quantitative pain measurement to improve pain studies and increase efficiencies through statistically significant outcome measures that support translational medicine.

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Validation Animal and Human Studies

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<th>SPECIES</th>
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<tr>
<td>CANINE</td>
<td>Rehab; dogs underwent orthopedic exam [palpation, range of motion, and gait analysis] conducted in clinic</td>
<td>25</td>
<td>Differentiate Pain, Discomfort, Triggers, and Spasms</td>
<td>n=309 P &lt; 0.001 (α=0.05) Sensitivity 90% Specificity 97%</td>
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<td>EQUINE</td>
<td>Pain Diagnosis: laminitis, fracture, labor, colic, other. Case series using gold standard for pain assessment</td>
<td>71</td>
<td>Veterinary diagnosis using gold std. (exam, gait analysis, thermography) Pain / No Pain</td>
<td>Sensitivity 100% Specificity 92%</td>
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<td>EQUINE</td>
<td>Laminitis: horses were followed for three months to evaluate presence of pain in relation to veterinary diagnosis</td>
<td>20</td>
<td>Veterinary diagnosis using gold std. (exam, gait analysis, thermography) Pain / No Pain</td>
<td>P &lt; 0.001</td>
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<td>CANINE</td>
<td>Anesthetized Tail Clamp; dogs were anesthetized and subjected to noxious stimuli under varied SEVO</td>
<td>10</td>
<td>Qualitative PainTrace Nerve Conduction BIS (EEG) compared to MAC No Movement</td>
<td>Sensitivity 87% Specificity 90%</td>
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<td>CANINE</td>
<td>Spinal Surgery: focus on recovery pain; analgesic; environmental enrichment</td>
<td>10</td>
<td>Pain Correlated With Analgesia</td>
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<td>CANINE</td>
<td>Osteoarthritis: Placebo vs. NSAID cross-over design</td>
<td>10</td>
<td>Qualitative PainTrace compared to Orthopedic Exam and QOL Scales</td>
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<td>HUMAN</td>
<td>Shoulder (Rotator Cuff): human patients underwent orthopedic exam conducted by surgeon while simultaneously self-reporting pain during shoulder movement/manipulation</td>
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<td>Quantitative PainTrace simultaneously with VAS Self-Report; 10 standardized orthopedic rotator cuff tests</td>
<td>R(64) = −0.743 P &lt; 0.001</td>
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<td>HUMAN</td>
<td>Lower Back Pain: human patients were followed over five consecutive in-patient visit measure pre-treatment and post-treatment pain</td>
<td>20</td>
<td>PainTrace collected simultaneously with VAS Deltas Pre and Post Treatment</td>
<td>R(182) = −0.504 P &lt; 0.001</td>
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