Agriculture Genome to Phenome Initiative (AG2PI)
2022 Seed Grants

An AI Toolkit for Video Phenotyping in Livestock

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Keywords:
Machine Learning, Locomotion, Livestock, DeepLabCut, Deep Phenotyping

1. Objectives & Aims: Lameness presents a major animal welfare concern and is a significant economic burden for the livestock industry [1]. For example, lameness costs the dairy industry alone around $52 million a year [2]. Current methods of assessing lameness, conformation and locomotion phenotypes are often plagued by a lack of repeatability and accuracy, yielding heritability values for lameness of just 0.01 and 0.22; indicating need for a more accurate phenotyping approach for locomotor traits [3-5]. Visual assessment, the most common approach, lacks repeatability [6], accelerometer and gyroscope methods alter natural gait patterns [7], and reflective 3D markers are not feasible in less tractable livestock systems where application of reflectors and utilization of multiple-camera detector arrays is impractical and costly [8]. StepMetrix technology in cattle has documented stance time and ground reaction force [9] but has yet to consider diverse locomotor phenotypes indicative of lameness like back posture [10]. This project will utilize a published machine learning package (DeepLabCut, DLC version 2.2b7 [11]) in combination with a custom gait analysis pipeline to produce quantitative locomotor phenotyping protocols specifically for livestock. Previous work demonstrated the deep neural network (DNN) approach employed by DLC can label landmarks on an animal with the same accuracy as the human eye but in far less time [11]. For example, the pilot project described below would take one full-time operator about four months of continuous work to label the 77,000 frames of data, but only a few hours by applying the DLC machine learning approach.

We have already conducted pilot experiments with beef cattle and horses to test the versatility of the software on different livestock. We observed small groups of beef cattle moving in a wide grass lane, and horses as they were presented for veterinary inspections mandatory under the regulations of the Fédération Équestre Internationale (FEI) for sport horse competition. For both species, we use a consumer level Sony α6400 camera shot set at 120 frames per second (fps) and 1280 x 720p resolution. Pilot work resulted in definition of our key landmarks on the cow (Figure 1a) and the horse (Figure 1b) and trained species-specific DNN networks to recognize these landmarks and label them accurately.

In our pilot dataset we select 20% of the total sample of videos for training, then DLC selects 20 random frames from each training video for manual labeling by the operator and based on these human-applied labels trains the model for over 1 million iterations on a single NVIDIA graphical processing unit (GPU). Computationally applied labels can be manually refined to improve accuracy, and the DNN is re-trained to reflect the improvements. The newly generated model will apply labels to all frames in the entire sample set. The completed DLC-based labeling process results in a .csv file recording the coordinates of every label in each frame of recorded video.

Although the DLC package rapidly accomplishes labeling of the video observations, each .csv table of coordinates must then be processed into human interpretable phenotypes. Our first draft pipeline runs in Python (Version 3.7.6) utilizing a Kalman filter for trajectory smoothing to remove spurious labels that were not corrected during manual refinement. A series of transformation approaches are used to produce the 11 draft gait parameters and standard deviations for each [12]. This application seeks to provide dedicated effort to improve this first draft computational analysis step of the procedure, enabling broad distribution of the approach in order to foster inter-institutional collaboration. We can then use online surveys and our existing virtual "Research Interest Group" meetings to strategize on additional traits to extract from the data, and prioritization of livestock systems for pilot projects. Ultimately, we aim to create the tools necessary to work make quantification of these phenotypes accessible and interoperable across a broad network of animal scientists. The generated diverse and highly quantitative locomotor phenotypes will enable genetic studies with importance to many livestock systems. Enabling future genomic selection tools and immediate applications in early detection of lameness will prevent monetary loss to the farm, improving overall livestock welfare. In the short-term, this application addresses the critical need for a computational toolkit that can be rapidly adapted for the complex task of locomotor phenotyping and applications in high-throughput precision management systems.

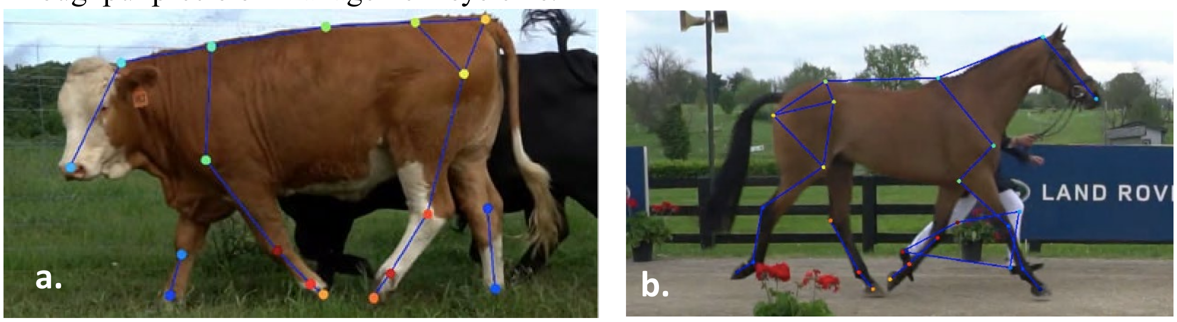

Figure 1. Example video frames of a beef heifer (a) and a sport horse (b) labeled with color-coded skeletal landmarks by DLC.

Aim 1: Develop Digital Labeling of Skeletal Landmarks in a Large Sample Size.

Veterinary inspections provide an ideal model for capturing a large sample set required for construction of a robust labeling model in livestock. From our database of 2,535 videos already captured, we trained the DLC model using 502 videos, then selected a sample of 94 videos observing 47 horses during their pre- and post-exercise mandatory veterinary inspections at a competition for labeling with this model. This observational experiment allows testing of the functionality of DLC and our custom gait analysis pipeline under realistic field conditions, assessing stride parameters altered by fatigue that were previously identified with standard animal-mounted equipment [13-15].

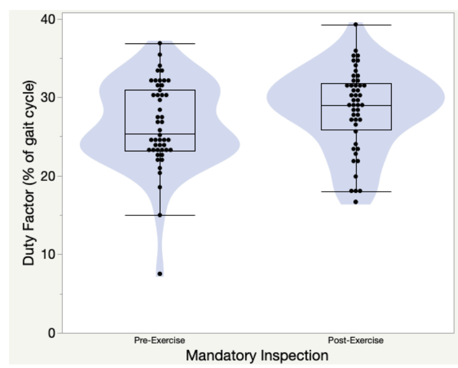

Figure 2. Digital video analysis captures a slight but significant change in the duty-factor (stance time/swing time) in the stride of fatigued horse (P = 0.0342).

Preliminary analysis of the pilot data set revealed that a significant increase in the “duty factor”, a ratio of the stance phase to the swing phase of the stride, post-exercise (P = 0.0342) (Fig. 2), and a trend toward a decrease in speed (P = 0.0624). While these animals are elite athletes and likely not representative of the general animal population, they do provide an opportunity to test the sensitivity of our approach; in this experiment documenting a change in the stride equivalent to a few milliseconds of extended stance phase.

Aim 2: Develop a custom analysis pipeline to process raw marker coordinates into locomotor parameters appropriate for livestock.

DLC falls short of providing a human-readable output of the interpretations for each individual video. DLC currently produces a large table containing coordinate positions of every labeled point. In the pilot study, the labeled 94 videos generated over 1,692,000 cells of individual X and Y coordinates to analyze. The draft pipeline, as explained in the methods, relies on a user-friendly code created in Python and MatLab. The DLC generated .csv files of label coordinates are reduced to a human-readable table containing the calculated locomotor parameters. This function is applied to each observable frame then mean and standard deviation summary values are calculated for each individual. We currently have 11 pilot gait parameters, but these just begin to assess locomotor traits in diverse livestock, and we will expand these based on the input of our collaborative network. At this point the custom pipeline is a critical need to even begin to capitalize on the hands-free DLC labeling approach and requires significant interdisciplinary collaboration with data and computer scientists to ensure a robust and broadly applicable tool that will be a resource to livestock scientists working in diverse fields.

Aim 3: Field test custom DNN models and the analysis pipeline in diverse livestock.

Livestock are herd animals often managed in extensive group settings. Thus, the resulting custom gait analysis pipeline will need to function with minimal human effort and in extreme environmental conditions. Preliminary data labeling 18 skeletal landmarks on video of 14 cows and yearling calves of various breeds allowed detection of longer relative limb length in the yearling calves compared to the adults, a common paedomorphic trait (Fig. 3). Additional video recording sessions conducted at the University of Florida dairy, beef, and sheep facilities, as well as those submitted by collaborators at several institutions, will strengthen the pipeline for analysis of diverse livestock. With the construction and broad sharing of these pre-trained labeling DNN models, we will improve the accessibility of our pipeline, eliminating the need for large RAM or GPU access, as well as expanding the utility of the toolkit to diverse species. Adaptations of the pipeline could also allow lower resolution and frame rates amenable to capture with a simple cell phone camera, providing global collaborative opportunities. The full pipeline and fully illustrated tutorials will be published as the work is completed on GitHub, as well as fully described in a scientific manuscript.

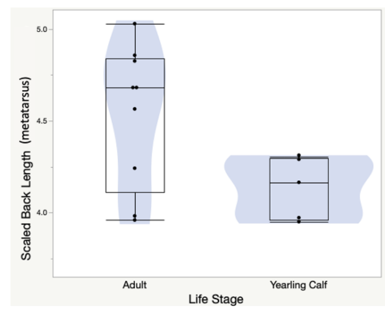

Figure 3. Pilot data documenting changes in body proportion in a mixed-breed herd of 14 cows and calves.

2. Furthering AG2PI Aims: The primary goal of the AG2PI goal is to span diverse scientific disciplines to prepare the research community for large-scale projects that will improve understanding of phenotypes valuable to agriculture. The proposed custom gait analysis pipeline addresses this aim by laying the foundation for a collaborative network focused on phenotyping of locomotion. Currently, there is a critical need for a broadly sharable tool to quantify the complex phenotypes of locomotor behavior in livestock. Distribution of the first draft pipeline will promote cross-fertilization of ideas, improving accessibility of work in this field and allowing expansion of a global collaborative network. In addition to the distribution of the method via GitHub, the findings will be disseminated through workshops, industry events, national and international scientific conferences, and local seminars at the University of Florida. Formalization of our collaborative network, expansion of our "Research Interest Group" meetings and community input through online surveys will also catalyze diverse research based on this critical toolkit.

3. Expected Outcomes & Deliverables: Project success will be measured by the number of views for the pipeline on GitHub, and the number of downloads, publications, and citations of our published work. In addition, the number of trainees, including undergraduate research volunteers, will increase within the provided educational and research opportunities. Development of this critical tool will also enable a variety of subsequent grant applications addressing various questions regarding locomotor phenotypes in livestock. Our project will provide, at a minimum the following short-term **outcomes and deliverables:**

- A toolkit of scripts built around the DLC method to quantify locomotor parameters.
- Initial pilot datasets utilizing AI-derived gait phenotypes in multiple species of livestock.
- Two scientific publications: 1) Methods paper describing the custom gait analysis pipeline 2) Application paper for the use of the custom gait analysis pipeline in livestock.
- Multiple presentation opportunities for undergraduate and graduate students at local, national, and international conferences.

4. Qualifications: The core team research team is based at the University of Florida. However, we collaborate broadly with a community of researchers hoping to soon utilize the resulting toolkit. **Dr. Samantha Brooks** brings expertise on precise and high-throughput phenotyping using diverse technologies like geometric morphometrics and metabolomics. Dr. Brooks mentors the Masters student on the project, Madelyn Smythe in collecting and processing video in the DLC software and analyzing pipeline outputs, as well as training of undergraduate researchers contributing to the project. **Dr. Kyle Allen's** research group brings to the collaborative team specific expertise in video as a tool for assessing limb function in rodent models of osteoarthritis and orthopedic pain. His approaches manage big data scale outputs from these tools, utilizing appropriate quality controls and stringent statistical tests. For this work, his group will adapting these draft pipelines for analysis of livestock labeling networks. **Dr. Biedrzycki** leads a research program focused on advanced diagnostic imaging, biomechanics and orthopedics in the horse, as well as tendon injury and rehabilitation. Dr. Biedrzycki will provide expertise in research and clinically relevant parameters for interpretation of abnormal limb function in large animals. Dr. Biedrycki also works closely with colleague **Dr. Joao Bittar,** specializing in beef cattle extension in the Large Animal Clinical Sciences department. Dr. Bittar brings specific expertise in field identification of locomotor traits in cattle.

5. Proposal Timeline: The new analysis pipeline should be completed in late spring 2022 (aim 1), new landmarks proposed by survey results added in fall 2022 (aim 2) and utilized in livestock field tests in fall 2022/winter 2023 for training of new models (aim 3). Once this first draft toolkit is established, it will form the basis for expansion of the work through collaborations with diverse institutions. This will not only diversify our current data set, but aid in building new trained networks on a variety of species including, but not limited to swine, goats, sheep, and dairy cattle. These future collaborations will be essential in expanding the tool for field use and further developing our list of detectable phenotypes.

6. Engaging Scientific Communities and Under-Represented Groups: Our collaborative research team often supports underrepresented groups in science through mentorships, internships, and assistantships for undergraduates in their first research experience. Tools like the pre-trained DNNs packaged with the custom pipelines, freely available on the internet, will also eliminate the need for expensive computational hardware, improving global accessibility of this method.

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