Research Notes

Use of thermography to screen for subclinical bumblefoot in poultry

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ABSTRACT Thermographic imaging is a noninvasive diagnostic tool used to document the inflammatory process in many species and may be useful in the detection of subclinical bumblefoot and other inflammatory diseases. Bumblefoot is a chronic inflammation of the plantar metatarsal or digital pads of the foot (pododermatitis), or both. It is one of the major health problems in birds including chickens and is responsible for significant economic losses in commercial poultry operations. Early diagnosis of bumblefoot is essential for the prevention of economical loss and the improvement of animal well-being. The object of this study was to determine the suitability of thermography for the identification of subclinical bumblefoot in chickens. Experiment 1 was designed to validate thermography as a tool for screening avian populations for bumblefoot. The plantar surface of the feet of 150 randomly selected hens was imaged using a thermal camera. The thermal images were identified as suspect, positive, or negative for bumblefoot based on thermal patterns of the plantar surface. Visual inspection of the feet identified as suspect followed 14 d later. A visual score of clinical, mildly clinical, or negative for bumblefoot was assigned, based on gross pathological changes in the plantar surface. A correlation between initial thermal images identified as suspect for bumblefoot and a visual score of positive 14 d later was 83% ($P < 0.01$). In experiment 2, hens whose feet were free of lesions were inoculated in the metatarsal foot pad with *Staphylococcus aureus*. Thermal images and visual clinical scores were taken, prechallenge and 1, 2, 3, 4, and 7 d postchallenge. The correlation between thermal images classified as clinical and a visual score of clinical for bumblefoot was 86.7% ($P < 0.001$). However, the correlation between the thermal images classified as mild (subclinical) and a visual score of mild was only 26.7%, suggesting that thermography is a more sensitive indicator of subclinical infection than visual appraisal. Thermography may thus provide a useful tool for screening avian populations for signs of bumblefoot, early in the course of the disease, which will improve recovery percentages and bird well-being.

Key words: bumblefoot, infection, thermography, chicken, welfare

2009 Poultry Science 88:1176–1180
doi:10.3382/ps.2008-00446

INTRODUCTION

Thermographic cameras are able to detect radiation in the infrared range of the electromagnetic spectrum and to convert this radiation into an image. The intensity of infrared radiation emitted by an object increases with temperature according to Planck's law of blackbody radiation. It provides both qualitative and quantitative information on the surface temperature of the target tissues (Maldague et al., 2001). Thermal imaging has been used in many animal species to document inflammatory processes associated with skin-surface temperature changes (Turner et al., 1983, 1986; Purohit, 2006) and to determine the physiologic reactions of challenges to the sympathetic nervous system (Heath et al., 2001). Thermographic imaging appears to offer a noninvasive, nonionizing diagnostic tool for detecting subclinical foot infections, such as bumblefoot, in birds. However, this hypothesis has not been tested.

Bumblefoot (pododermatitis or foot pad dermatitis) is a chronic inflammation characterized clinically by abrasion, ulceration, and swelling of the plantar metatarsal or digital pads, or both, in birds. Bumblefoot causes pain, impedes perching and walking, and may limit access to food and water (Hester, 1994). If left untreated, bumblefoot will compromise the internal tissues of the foot, such as the mesoderm, tendons and bones, causing osteomyelitis, synovitis, laminitis, and eventually death (McNamee and Smyth, 2000). It occurs in captive avian species from raptors to penguins (Rodriguez-Lainz et al., 1997; Reidarson et al., 1999; Tarello, 2002) as well as in domestic poultry (Abra-

Received October 15, 2008.
Accepted January 26, 2009.
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hamsson et al., 1996; Barbour et al., 1997; McMullen, 2004). Sea World (San Diego, CA) reported a bumblefoot incidence rate of 64% in the captive penguin population (Rodriguez-Lainz et al., 1997). In poultry, bumblefoot was found mostly in the chickens maintained in floor housing systems but not in those caged (Tauson et al., 1999). In a survey conducted by Martrenchar et al. (2002), about 40% of 50 flocks of chicken broilers were of bad quality and more than 10% of those birds had severe foot lesion (score 3: lesion on >50% of the pads). In the poultry industry, bumblefoot causes economic losses due to field rejection (Shane, 2007), carcass rejection, and poor growth due to lameness (Hester, 1994).

Increased incidence of bumblefoot is associated with wet litter, usually found in floor-housed chickens (Martland, 1984; Martrenchar et al., 2002). In caged laying hens, increased incidence of bumblefoot is associated with pressure on the metatarsal foot pad due to perch design (Oster, 1994; Tauson, 1998) and wet or unhygienic perches (Tauson and Abrahamsonn, 1996). Nutrient deficiency (Burger et al., 1984) is also a known contributor to increased incidence of bumblefoot in laying hens. The rate of bumblefoot incidence per farm is gaining recognition as a well-being indicator (Martrenchar et al., 2002).

The etiology of bumblefoot involves bacterial components. *Staphylococcus aureus* has been cultured from 90% of the spontaneously occurring cases of bumblefoot, depending upon avian species and environment (Satterfield and O'Rourke, 1981). *Staphylococcus aureus* is a ubiquitous gram-positive bacterium present in high concentrations in the dust of poultry houses, animal feed, and gut contents and even on the skin of nonclinical animals (Cotter and Taylor, 1987; Zhu et al., 1999). When the mucosal or skin barriers have been compromised due to trauma or stress, *S. aureus* can invade the mesoderm and proliferate and cause inflammation. Early diagnosis and treatment prevents complications caused by lameness and are essential for a positive prognosis. The object of the present study was to validate thermal imaging as a diagnostic tool for detecting subclinical bumblefoot in chickens.

MATERIALS AND METHODS

All of the following experimental protocols were approved by the Purdue Animal Care and Use Committee.

Birds

Both studies utilized 60-wk-old White Leghorn W-36 hens, which were used in another study. In that study, the hens were housed in 4-bird cages up to 60 wk of age. Each cage provided 72 in^2 (0.046 m^2) per hen. In the current study, the hens were randomly housed in 2-hen cages (experiment 1) or single-hen cages (experiment 2) at the Purdue University Poultry Farm or a facility of the School of Veterinary Medicine, respectively. Each cage provides 72 in^2 (0.046 m^2) per hen. Water and a standard layer ration were provided for ad libitum consumption. Overhead lights were under a 16L:8D cycle.

Imaging

To capture thermal images, each hen on study was held upside down by the hocks with the dorsal side of the feet pressed against a wall (Figure 1). Thermal images of the ventral view of the foot were taken using a FLIR ThermaCAM PM695 (FLIR Systems Inc., Boston, MA) mounted on a tripod at 0.91 m from the wall. Digital images were captured simultaneously using a Sony NCV-FD83 (Tokyo, Japan) also mounted on a tripod at 0.91 m from the same wall.

The thermal images, from both experiments, were analyzed using Reporter 2000 software (FLIR Systems Inc.). The thermal pattern for the ventral view of a sound avian foot shows a decrease in temperature from the metatarsal foot pad to the digit extremities of less than 5.8°C (±1.9; Figure 1A). This thermal pattern should correspond to the dissipation of heat as the blood flows from the central foot pad to the periphery, with the decrease in heat as the increase of distance from the blood vessels.

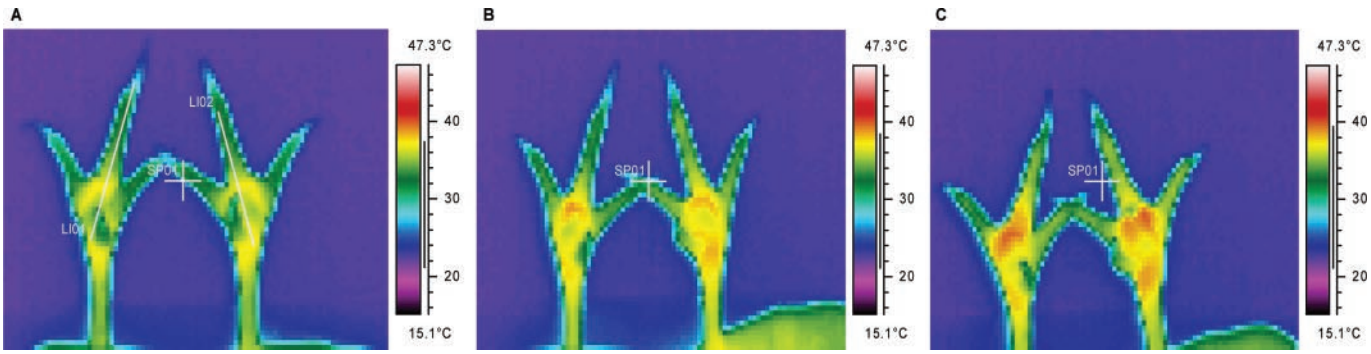

Figure 1. Thermal images of the feet of hens. A typical thermal image of the feet of hens classified as (A) S (sound or negative), (B) MC (mildly clinical bumblefoot or suspect), and (C) CL (clinical bumblefoot or positive).

Visual Score

The feet of the hens were visually scored for signs of bumblefoot. Any foot with pustules or swellings that were visible at first glance was scored as clinical (**CL**). Any foot that looked red, slightly swollen, scabbed, or caused the inspector to reexamine the foot was scored as mildly clinical (**MC**), and any foot that presented no visible anomalies was scored as sound (**S**).

Validation of Thermal Imaging in Chickens, Experiment 1

One hundred fifty hens were randomly picked and assigned to the current study from a population of 320 hens. On d 1, thermal images of the plantar surface of the feet of each hen were captured. Based upon the thermal pattern of the thermal image, the feet were classified as S, MC (suspect), and CL (positive) for bumblefoot. Images that showed no unusual thermal patterns were classified as S (Figure 1A). Images classified as MC showed small areas of unusual heat patterns (Figure 1B), and those classified as CL for bumblefoot showed definite large areas of abnormal heat patterns (Figure 1C). Fourteen days after the initial imaging, the feet classified as MC were visually scored again for signs of clinical bumblefoot.

Validation of Thermal Imaging in Experimental Bumblefoot in Chickens, Experiment 2

Forty-two hens, whose feet were visually inspected and determined to be free of lesions, were selected and randomly assigned to 3 treatments. These hens were housed individually in standard battery cages in a level II biological containment room (School of Veterinary Medicine, Purdue University). Test hens (n = 30) were given a 0.5-mL s.c. injection containing 5.3×10^7 cfu/mL of *S. aureus* in each metatarsal foot pad. Control hens were given 0.5 mL of saline (n = 10). Growth medium was not used to minimize a possible inflammatory response to the medium. Two hens were not injected at all to serve as a negative control against possible effects of the saline injections.

Preparation of Staphylococcus Inoculum

Lyophilized *S. aureus* (ATCC no. 29506) was revived in 0.5 mL of bovine-yeast-peptone (**BYP**) broth (g/L; beef extract, 10 g; peptone, 10 g; yeast extract, 3 g; NaCL, 5 g; Difco, Detroit, MI) and incubated at 37°C overnight. The inoculum was enumerated by serial dilution in BYP and grown overnight at 37°C on 15% agar plates. The optical density (**OD**) of each dilution was measured (Spectronic 70, Bausch and Lomb, Surrey, UK). The final inoculum was diluted to an OD of 0.16 with BYP for the challenge (see above). Preliminary experiments indicated that this OD was equivalent to 5.3×10^7 cfu/mL.

Data Collection

Thermal and digital images were captured before inoculation and then at 1, 2, 3, 4, and 7 d after inoculation. On d 7, the feet of each hen were inspected and given a visual score of CL, MC, or S.

Image Analysis

On each image, a line was drawn using the Reporter 2000 analysis software (FLIR Systems Inc.) from the middle toe across the metatarsal pad for each foot. The temperature differences (**TD**) between the maximum and minimum temperatures along that line were calculated. A score of S was given to a foot with a TD of 7.7°C or less throughout the study. A score of MC was given to any foot with a peak TD between 7.7 and 9.2°C, and a score of C was given to any foot with a peak TD above 9.2°C at any time during the study.

Statistical Analysis

Statistical analysis for both experiments was done using SAS software (SAS Institute, 1992). Each data set was tested for normality. Transformation was not necessary. The correlation between the categories of the thermal images and visual scores for both experiments was analyzed using SAS regression analysis. The ANOVA of the mean TD (**MTD**) in experiment 2 was analyzed using 1-way ANOVA to examine *S. aureus* challenge in inducing bumblefoot. When a significant difference was obtained ($P < 0.05$), the differences between treatment within each single time frame were tested using post hoc paired *t*-test. Statistical significance was at $P < 0.05$.

RESULTS AND DISCUSSION

Based upon the initial thermal images, 25 of the 150 hens in experiment 1 were scored as CL, 43 hens were MC, and 77 hens were S. Five hens were not classified because the images were not sufficiently clear (data not shown). Of the 43 MC hens, 36 hens received a visual score of CL at 14 d later. The correlation between the image-category and the visual score for bumblefoot was 83% ($P < 0.01$).

Previous studies have reported that the most cases of bumblefoot were found in broiler chickens and turkeys that are housed in floor pens and a few cases were found in the chickens housed in cages (Tauson et al., 1999; Martrenchar et al., 2002). One of the major causes of bumblefoot is inflammation of the skin due to corrosive factors present in wet litter (Tauson et al., 1999; Martrenchar et al., 2002). The reasons for the high incidence of bumblefoot in the present study are

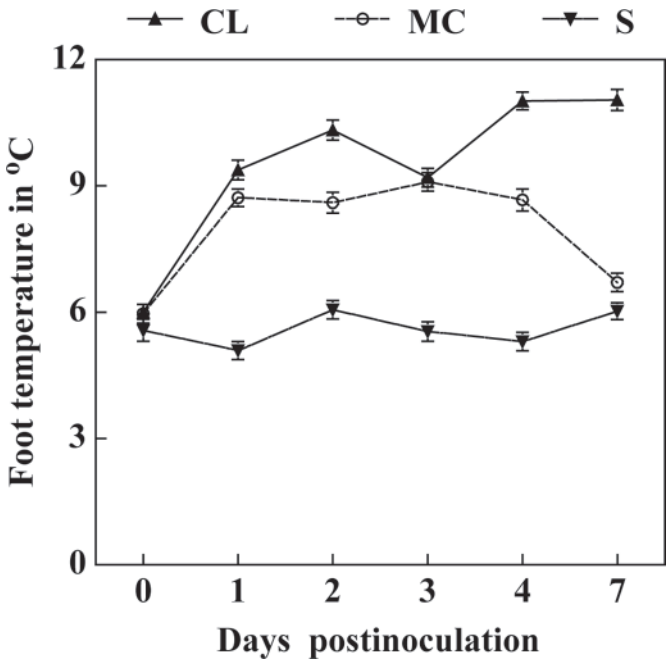

Figure 2. Change in mean temperature of the metatarsal foot pad of hens after *Staphylococcus aureus* challenge. Treatment hens were inoculated in the metatarsal foot pad of each foot on d 0 and thermal images were taken on d 0, 1, 2, 3, 4, and 7 postinoculation. Based upon the peak temperature difference between the toe and the metatarsal foot pad, the images were classified as S (sound or control), MC (mildly clinical bumblefoot), and CL (clinical bumblefoot). Data were presented as the means ± SD for each day (n = 10 for controls and n = 30 for inoculation).

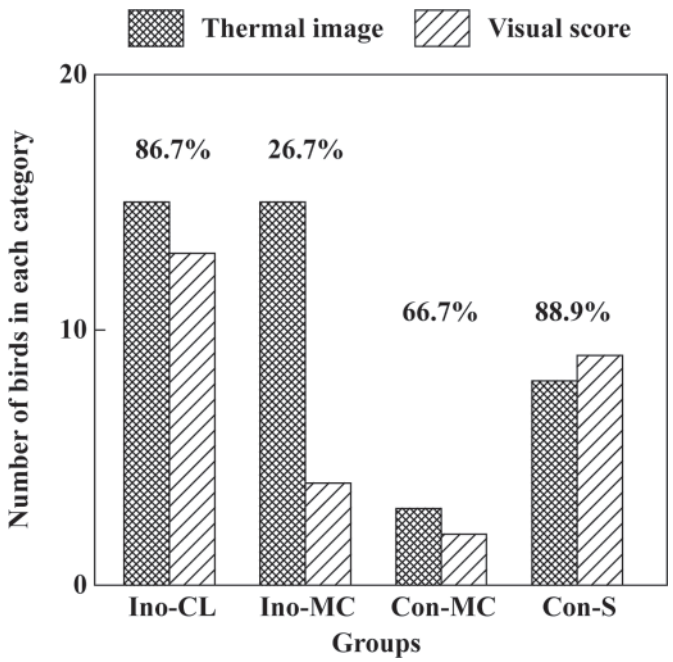

Figure 3. Visual and thermal image scores in diagnosis of bumblefoot after *Staphylococcus aureus* challenge. When a thermal image was taken, a visual score was assigned to each foot based upon appearance and severity of lesions during the 7 d postinoculation (n = 10 for controls and n = 30 for inoculation). There was a significant correlation between visual score and the thermal images in the hens classified as having clinical infections ($P < 0.001$), whereas the correlation between the hens classified as having mildly clinical infections by visual score and thermographic image was only 27% ($P < 0.001$). It may indicate that approximately 73% of the cases examined visually were incorrectly classified as negative infection for bumblefoot. Con-MC = mild clinical cases in the control group; Con-S = sound cases in the control group; Ino-CL = clinical infected; Ino-MC = mild clinical infected.

unclear, but it could be related to the effects of previous study (furnished cages vs. battery cages). In the previous study, a particularly high incidence of bumblefoot was found. It may be due to perches and claw shortener used in that study. However, it does not affect the aim of this study (i.e., thermographic imaging may be a useful tool for screening avian populations for signs of bumblefoot and foot infection).

The MTD for the control hens in experiment 2 was 5.8 ± 1.9°C, with no significant difference between days during the observation period ($P = 0.38$). The MTD for the *S. aureus*-treated hens (8.8 ± 2.1°C) was significantly higher than that of the controls over all postinoculation days ($P < 0.05$). The MTD of hens of images classified as CL and MC followed the same trend of increasing temperature until d 3 postinoculation. After d 3, the trend diverged with the MTD of the images classified as CL increasing to an MTD of 9.2°C, whereas the MTD of the images classified as MC decreasing, approaching the levels of the controls by d 7 (Figure 2). The later change may indicate recovery in those birds.

The correlation between the thermal images and visual scores at d 7 postinoculations was 86.7% for the hens classified visually as CL (Figure 3, $P < 0.001$). However, the correlation between the hens classified as MC by visual scores and thermal images was 26.7%. These findings may indicate that approximately 73% of the cases examined by visual inspection incorrectly classified infected hens as negatives for bumblefoot. The results may suggest that visual inspection is not sensitive enough to detect early stages of infection.

The present data evidence that thermography has the ability to detect bumblefoot even at the subclinical level missed by visual inspection, and it can be used as a tool for screening avian populations. Interpretation of thermal patterns of the avian foot as well as changes in surface temperature not only revealed subclinical bumblefoot but also helped to evaluate its severity and to monitor the progression of the disease. Early detection and easy monitoring may improve prognosis and decrease associated pain, which improves animal well-being.

ACKNOWLEDGMENTS

We thank the employees of the Animal Research Center of the College of Veterinary Medicine at Purdue University for their assistance in the collection of data.

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