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DETERMINING A PAIN CAUSED BY FOOT ROT DISEASE IN GOATS USING FACIAL EXPRESSIONS, PERIPHERAL TEMPERATURES AND CORTISOL HORMONE

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Abstract

No study is undertaken to establish a face grimace scale for goats. Therefore, the present study was designed to measure facial expressions, peripheral temperatures and cortisol hormone of goats affected with foot rot disease. Forty goats (20 healthy and 20 infected) were used in this study. Two images were captured from each animal to detect facial grimace scale of goats. Eye, ear and nasal temperatures were measured using infrared thermal camera. In addition, cortisol hormone was obtained from blood serum. Results revealed that there was a significant drop ($P<0.01$) in eye, ear and nasal temperatures of infected goats compared to healthy animals. In addition, cortisol hormone was significantly ($P<0.01$) higher in infected animals at day 1 and day 7. The changes in facial expressions were significantly ($P<0.01$) different than healthy animals, except for the cheek (masseter) muscle. The total pain score was significantly ($P<0.01$) higher in day 1 and day 7, compared to healthy animals. Sensitivity of facial grimace scale was 0.75 and 1-specificity was 0.35. It was concluded that using changes in facial expressions in goats is a good tool for detecting pain.

Keywords: Cortisol, Foot Rot, Facial Expressions, Goats, Pain, Peripheral Temperatures

1. INTRODUCTION

Foot rot is a contagious disease in ruminants, including goats. This disease causes not only the production loss but also economic loss [1]. Foot rot destructs the hoof hard-layered keratin that leads to lameness in many cases. This disease can be characterized by inflammatory exudates, foul smelling, as well as necrosis of the hooves epidermal tissue [2]. Most of diseases, including foot rot, causes pain to animals [3].

Many diseases that affect ruminants cause distress as a result of their pain level. As a prey animal, sheep and goats have a habit of not expressing the pain signs openly [4]. There is yet lack of recognizing these pain signs of animals, such as goats, by human [5] that is mainly related with some diseases like mastitis, foot rot and pregnancy toxemia [3, 6, 7]. Therefore, both pain quantification and identification are essential for successive treatment of the disease and pain relief [8].

Both physiological and behavioral indicators can be used to measure pain in animals. Regarding the physiological indicators, temperature and cortisol hormones are

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commonly used. Both cortisol hormone and body temperature are increased when animals suffer pain, in turn, the surface temperature like eye temperature is decreased [9,10,11].

Rectal temperature is frequently used to measure the core body temperature of animals. Although, it can lead to stress or injury to animals and/or human [12]. For that reason, a non-invasive method using infrared thermal imaging to indicate surface temperature is more appropriate [13,14]. Some regions are commonly used to measure surface temperatures, for instance, eyes, ear and nasal. However, eye temperature has indicated the most reliable results [15]. The emissivity, which is the ability of object to radiate infrared energy, may affect surface temperature measurement [16]. The emissivity of most animals is between 0.86 and 0.98 [15]. Thus, the emissivity of thermal camera must be checked in order to avoid its effect on the measurement. The hypothalamic-pituitary-axis is stimulated during both negative and positive states of animals, which in turn causes an increase in catecholamine and glucocorticoid thus causing more heat loss. The heat loss can be detected by a drop in surface or peripheral temperatures [17,18,19]. A previous study obtained that the peripheral temperatures were significantly decreased after ear tagging procedure in kid goats [11]. As an indicator of pain in farm animals, cortisol hormone is widely used such as in cattle, pigs, and sheep [20,21,22]. Cortisol samples were mostly collected from blood serum [23]. In addition, in recent years, there is a growing interest in measuring facial expressions of several animals as pain indicator.

McLennan et al. [3] introduced a scale for facial expressions in sheep, which provided a reliable tool for assessing pain caused by diseases like mastitis and foot rot. This scale analyzed five interested regions of head to determine pain level, including ears, nose, cheeks, eyes and lips. The scale was highly accurate not only in pain identifications, but also in identifying illness signs. There is still lack of such scale in goats. It was reported that there is an urgent requirement for validating a face grimace scale for goats [24]. Therefore, the present study was designed to establish facial expressions, measuring peripheral temperatures and cortisol hormone of goats affected with foot rot disease.

2. MATERIALS AND METHODS

2.1 Study Area and Subjects

A study was undertaken in Batifa District villages from November 2021 to July 2022. Twenty healthy and 20 infected goats with foot rot were included in this study. All animals were treated by a specialized veterinarian.

2.2 Ethics Approval

The procedure of this study was approved ethically by the Animal Ethics Committee of the Faculty of Sciences, University of Zakho, Kurdistan Region – Iraq with its approval code: ARC015. All animals were normally infected with foot rot and were treated carefully.

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2.3 Data Collection

2.3.1 Facial Grimace Scale

Images were taken from side and front of the head of each ewe for every stopwatch using a high resolution Sony Camera (SONY, Cyber-Shot, DSC-H20, Japan). Two pictures were taken from each goat, i.e 2 pictures for each healthy subject and 4 pictures of infected goats in 2 times (day 1 and day 7) with total of 120 pictures. The pictures were cropped to include head only and were scored 4 months after the procedure so as to exclude any bias when scoring face actions. These facial units were measured: orbital tightening, ear position, lip and jaw profile, cheek tightening, and nostril and philtrum shape. If the authors were not able to score 2 or more regions of face, the picture was removed from total score of pain. Thus, the total of 18 pictures were excluded. The 3-scale score was used to score the facial units (0 = not present; 1 = moderate; 2 = severe). (For details, see McLennan et al. [3])

2.3.2 Temperature Data

Peripheral temperatures were collected from eye, nasal and ear using Infrared Thermal Camera (FLIR E4, FLIR Systems, OU, Estonia). Picture were captured from a distance of half to one meter as previously done by researchers [25]. images were containing the measured area temperature on the left top corner from the ball in the image shows the place of measuring area.

2.3.3 Cortisol hormone

The blood samples were collected from jugular vein of goats by a specialized veterinarian. At the beginning, an animal was restrained by the animal owner. Then, a veterinarian applied a sterile syringe (HiGeen, PRC) to collect 5 mL of the blood and placed in the test tube. The blood samples were centrifuged at the laboratory in Batifa district at 2000 rpm for 5 minutes. The serum was then removed from the centrifuged tubes with Pasteur pipets and placed it in a new tube. The tubes containing serum were placed in a freezer for later analysis. The procedure of collecting blood samples was first done for healthy goats. Then, twice for infected goats with foot rot at the beginning of infection (Day 1) and 7 days later for same animals (Day 7). At the laboratory, cortisol hormone was determined from the serum using Cobas Elecsys Analyzer (Roche Diagnostics, Germany).

2.4 Data Analysis

All data were recorded in a new Microsoft Excel spreadsheet. Data were prepared and projected to GenStat software program (17th edition, VSN International) so as to be analyzed. Unless where otherwise stated, all the results were recorded as mean ± SEM (standard error of mean). Shapiro-Wilk normality test discovered that both cortisol and temperature data to be parametric, whereas all other data face expressions to be non-parametric. Thus, for cortisol and temperature data, ANOVA one-way repeated measures was used with Fisher's Unprotected LSD test for post-hoc comparisons. For non-parametric data, Kruskal Wallis test was used following by Mann-Whitney U-test for

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post hoc comparisons. All tables and figures were arranged in Microsoft Excel spreadsheet. Sensitivity (the true positive's ratio) and specificity (the true negative's ratio) were scored.

Sensitivity = TP / TP + FN

Specificity = TN / TN + FP.

Where:

TP = true positives (sick goats identified as sick)

TN = true negatives (healthy goats identified as healthy)

FP = false positives (healthy goats identified as sick)

FN = false negatives (sick goats identified as healthy)

3. RESULTS

3.1 Temperature

The temperature of the goats in day 1 of infection was significantly different in comparison to the healthy goats and infected animals at day 7 (Table 1). Regarding the eye temperature, the infection (Day 1) caused a drop by 4.4 °C. However, the eye temperature was increased again at day 7 by 3.3 °C. Similarly, the ear temperature dropped in infected animals at day 1 by 4.3 °C and increased after the treatment by day7. Lastly, there was a decrease by 3.8 °C in nasal temperature at day1 compared to healthy animals, and this temperature was increased by 3.0 °C in day 7.

Table 1: the temperature of eyes, ears and nose of the goats

Body Regions	Healthy animals	Infected animals		P value
		Day 1	Day 7	
Eye	37.5 ± 0.4a	33.1 ± 0.7b	36.2 ± 0.3a	0.01
Ear	33.5 ± 0.4a	29.2 ± 0.4b	32.8 ± 0.4a	0.01
Nasal	33.7 ± 0.6a	29.9 ± 0.6b	32.9 ± 0.5a	0.01

Note: different letters in the same row means significantly different.

3.2 Cortisol Hormone

Results of cortisol hormone showed that the cortisol hormone was significantly ($P<0.01$) increased in infected goats with foot rot in both day 1 and day 7, compared to control (healthy animals). The cortisol hormone levels for healthy, day 1 and day 7 were 11.66 ± 1.5, 45.01 ± 4.7, and 21.72 ± 2.8 nmol/l respectively (Figure 1).

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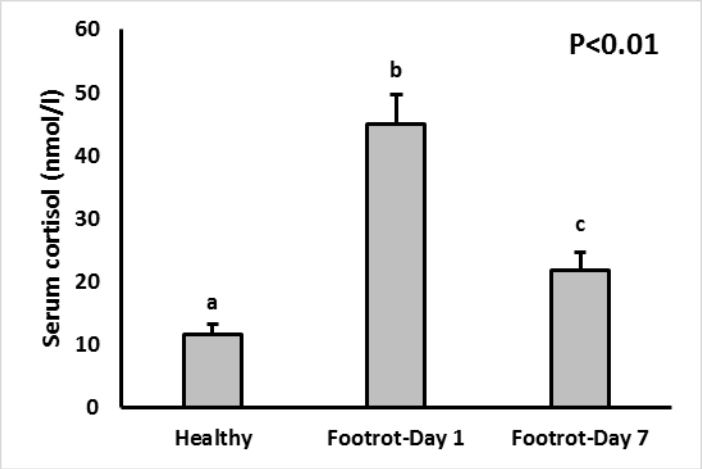
Figure 1: the levels of serum cortisol hormone from healthy and infected goats with foot rot



Note: different letters mean significant difference (P<0.01)

3.3 Facial Expressions

Results obtained from data analysis indicated that there were significant differences in all facial expressions, except the cheek (masseter) muscle, between healthy and infected goats with foot rot (Table 2). No significant differences were found between infected animals in day 1 and day 7.

Table 2: facial expression regions scored from 0 – 2 of healthy and infected goats

Facial Regions	Animal State			P-value
	Healthy	Foot rot Day 1	Foot rot Day 7	
Orbital Tightening	0.29 ± 0.11 a	1.41 ± 0.15 b	1.00 ± 0.14 b	0.01
Lip and Jaw Profile	0.35 ± 0.11 a	1.00 ± 0.12 b	0.77 ± 0.13 b	0.05
Nostril Shape	0.23 ± 0.10 a	1.35 ± 0.17 b	1.06 ± 0.15 b	0.01
Ear Position	0.59 ± 0.12 a	1.29 ± 0.14 b	0.88 ± 0.15 ab	0.01
Cheek Masseter Muscle	0.47 ± 0.12 a	0.76 ± 0.13 a	0.76 ± 0.16 a	n.s

In addition, there was a significant difference (P<0.01) in total pain score between infected and healthy goats. However, no significant difference was found between infected animals in day 1 and day 7 (Figure 2).

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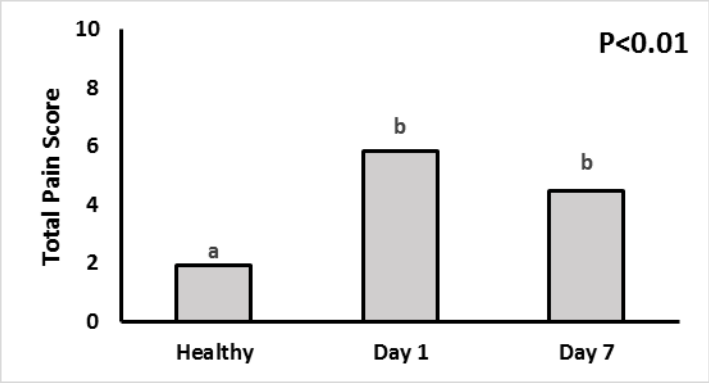
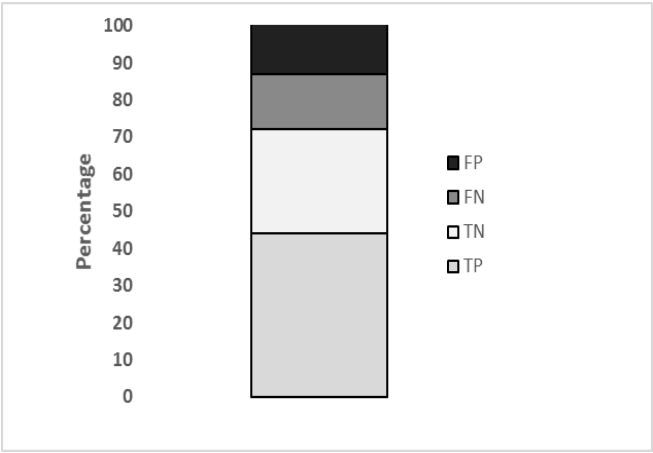
Figure 2: the total facial score of pain in healthy and infected animals at day 1 and day 7



Note: different letters mean significant difference (P<0.01)

The sensitivity or an ability of indicating true positive ratio was 0.75, whereas 1-specificity or ability of indicating true negative ratio was 0.35. high value of sensitivity indicates a high percentage of goats identified as positive for foot rot. A lower 1-specificity value indicates that higher percentage of goats were identified correctly as healthy.

Figure 3: the ratio of sensitivity and specificity values of true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN)



The correlations between each face area are shown in Table 3. The highest correlation was found in healthy animals between orbital tightening and lip and jaw profile (0.87). in addition, there was a high correlation between orbital tightening and lip and jaw at day 7 of infected goats.

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Table 3: the correlation between the five different facial regions of healthy and infected goats. The top row of each face area is of healthy animals, the middle row is for Day 1 infected goats and the bottom row is for Day 7 infected goats with foot rot

Face regions	Orbital	nostril	ear	cheek	lip and jaw
Orbital	-	0.25	0.28	0.43	0.87
	-	0.22	0.16	0.12	0.20
	-	0.18	0.17	0.48	0.73
nostril	0.25	-	0.18	0.31	0.17
	0.22	-	0.49	0.38	-0.18
	0.18	-	0.40	0.21	0.25
ear	0.28	0.18	-	0.07	0.12
	0.16	0.49	-	0.22	-0.43
	0.17	0.40	-	-0.06	0.10
cheek	0.43	0.31	0.07	-	0.29
	0.12	0.38	0.22	-	0.00
	0.48	0.21	-0.06	-	0.23
lip and jaw	0.87	0.17	0.12	0.29	-
	0.20	-0.18	-0.43	0.00	-
	0.73	0.25	0.10	0.23	-

4. DISCUSSION

The main objectives of the present study were to investigate the effect of foot rot disease on goat's welfare. The results obtained showed the significant drop in surface temperature of the infected goats. In addition, higher cortisol levels were determined from infected animals when compared to healthy goats. The total pain score of face expressions were significantly higher during the infection period.

The goats that had an infection with foot rot showed elevated serum cortisol levels, however these levels were decreased after the infection by 7 days of cortisol measurement and yet higher than the healthy goats' cortisol. According to Häger et al. [26] the salivary cortisol levels of sheep one-day post-surgery were increased and returned to baseline levels after 7 days. However, it was claimed that their number of animals were low. In the present study obtained similar results with higher number of animals; although, after 7 days the level of cortisol from serum was higher than the baseline of healthy animals. Cortisol levels were found to be increased in dairy calves after the procedure of disbudding [27]. In addition, cortisol was increased in sheep post-shearing [28]. Therefore, the present study result is consistent with the previous findings.

As underlined by the results of cortisol hormone levels, the authors established the goats grimace scale detect pain and distress in infected goats. The analysis of goat's facial expressions revealed a significant increase in the score of face expressions, except the masseter muscle of the cheek on day 1 and day 7 (Table 2). Within the day

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7, the mean face scores decreased but higher than the control (healthy goats). Similar results were found on sheep grimace scale, as the total sheep grimace scale was increased and the decreased by day 7 post-surgery [26]. It was found that total facial expression pain score was increased in sheep infected with foot rot, and in other species regarding the pain [3,26,29,30]. The pain caused by common husbandry procedures such as disbudding caused significant changes in facial expressions in kid goats [31]. In addition, lambs showed differences in orbital tightening and features of moth after tail docking [31]. The face expressions showed a high accuracy of this study. The sensitivity and specificity were determined in the present study as 0.75 and 0.35 for sensitivity and specificity, respectively. The TP of this study was 43.1%, TN was 27.5%, FP was 14.7 and FN was 14.7. The results of the present study are in line with the previous study of Häger et al. [26] who found FP was 22.7% and FN was 9.1%. with TP was around 40% and TN was less than 30% (accuracy of TP and TN was 68.2%). In addition, in their study. The accuracy of horse grimace scale was found slightly higher than this study by Dalla Costa et al. [32], which was higher than 70% and for laboratory mouse grimace scale was around 97% [33]. Precious study of McLennan et al. [3] obtained higher sensitivity and lower 1-specificity for sheep pain grimace scale. These results of higher sensitivity and lower 1-specificity reflect an assessment of pain that is careful and have fewer mistakes. The present scale of facial expressions in goats provides a reliable and accurate method for recognizing and assessing pain in goats. In addition, it can be used by farmers and veterinarians to understand about facial expression changes of goats during pain suffering. This can be achieved by changes in facial expressions because these changes are involuntary response to pain by animals [33] and leads to the higher sensitivity in pain evaluation [3].

Measuring surface temperature, significant differences were found in all measured areas (eye, ear and nasal) were found. In a previous study by Zebari et al. [11] showed that there was a significant drop in both eye and ear temperatures of kid goats following ear tagging procedure. Using negative stimuli in rabbits indicated that there was a decrease in both ear and eye temperatures significantly [34]. Following different stressful processes of handling, a significant decrease was found in the eye temperature of cows [17]. Similarly, when cows fed inedible feed, their peripheral temperatures were decreased [18]. It was shown that peripheral temperatures are dropped following both negative and positive states of animals [19,22]. However, different results to the current and previous studies were found by Arfuso et al. [28] who found a decrease in total eye temperature in sheep following shearing procedure.

5. CONCLUSIONS

According to the results obtained, it could be concluded that measuring peripheral temperatures and cortisol hormone are indicators of pain in goats. Most importantly, facial expressions of goats are useful tool to indicate pain. Using face grimace scale for goats is established in the current study. It showed high accuracy in obtaining diseased animals. More research is required to develop goat face grimace scale and farmers and veterinarians can rely on them for detecting early pain in goats.

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Conflict of interest

None

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