

The incidence of pododermatitis on broiler carcasses: lesion characteristics and sensitivity of acetic acid on associated bacteria

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Abstract: Broiler chicken feet are used as food and in the production of gelatin in many countries. Pododermatitis is a common disease in broiler chicken farming, which can affect the quality of broiler feet and be a perennial source of pathogenic bacteria that threaten public health. This research aimed to describe the lesion characteristics (macroscopic and histopathological) of pododermatitis in slaughtered broiler chickens, identify the associated pathogenic bacteria, and evaluate their sensitivity to acetic acid. One hundred thirty-six samples of pododermatitis lesions were subjected to morphological examination, and 129 underwent bacteriological examination to isolate and identify pathogenic bacteria using standard microbiological and biochemical tests. All isolated bacterial strains were tested for sensitivity to acetic acid using the agar dilution method. Pododermatitis samples of different scores underwent histopathological analysis. The results showed that score 4 was the most common (37.5%) for pododermatitis with an area of 2.91 ± 2.00 cm². Pododermatitis scores 3 and 4 showed the same microscopic lesions, including full-thickness epidermal ulcerations. Bacteriological analysis revealed that all 129 pododermatitis samples were positive, and 184 bacterial strains were isolated and identified. *Staphylococcus aureus* was the most frequently isolated bacterium (66.30%), and the second- and third-most prevalent were *Escherichia coli* (19.57%) and *Staphylococcus hyicus* (5.98%), respectively. Acetic acid was particularly effective against all tested bacterial strains (*Staphylococcus aureus*, *Staphylococcus hyicus*, *Escherichia coli*, *Salmonella enterica*, *Shigella flexneri*, and *Shigella sonnei*), with an average minimum inhibitory concentration (MIC) of $\leq 0.08\%$. In conclusion, broiler pododermatitis was a dominant disease, and *S. aureus* was the most important pathogen associated with pododermatitis in broiler chickens. Acetic acid was an effective product for controlling the bacteria involved in pododermatitis.

Keywords: Pododermatitis, lesions, incidence, broiler chickens, bacteria, acetic acid.

1. Introduction

In recent years, the poultry industry has experienced remarkable growth, resulting in a significant increase in the production of broilers and white meat at affordable costs for consumers. The marketing and use of broiler feet in human food are widespread practices in many cultures around the world. As a source of protein, chicken feet are also prized for their nutritional value (Taufik et al., 2010). However, it is essential to emphasize the importance of decontaminating the feet to ensure their safety. Consumers are becoming increasingly aware of these issues, thus favoring a more responsible approach to the marketing and use of chicken feet. Chicken feet are also used as raw material in the production of gelatin (Tiona and Rahelivololoniaina, 2023). Gelatin is widely used in the food industry to thicken, gel, and stabilize various products such as confectionery, desserts, yogurts, and even some savory dishes. This use of chicken feet helps minimize waste by efficiently harnessing animal resources to produce versatile ingredients in various culinary and industrial applications.

Pododermatitis, also known as foot pad dermatitis, is a disease characterized by necrotic lesions on the plantar surface of the foot pads of growing broilers (Jacob et al., 2016). Symptoms of pododermatitis include hard, scaly foot pad skin, abnormal keratin horn-like ankles, swollen and frequently splitting foot pad, and necrotic lesions of the epidermis that often split down the center of the lesion (Mayne, 2005). This disease is prevalent in broiler farms and has been reported in several countries. It poses a challenge to the modern poultry industry, as it is one of the most detrimental locomotor disorders affecting broiler welfare and a serious economic problem for broiler production (Szafranec et al., 2022). Pododermatitis also alters behavior and serves as a port of entry for pathogens, especially litter bacteria, which are in constant contact with the footpads. Many factors can contribute to the development of pododermatitis, including broiler feed, skin structure, body weight, sex, humidity, and litter type. However, wet bedding is the factor most likely to affect pododermatitis (Mayne, 2005).

Acetic acid is a well-established agent as an antiseptic, disinfectant, and food preservative, long recognized for its antimicrobial potential. Its use in controlling bacteria associated with pododermatitis in broiler chickens, whether on farms, in poultry slaughterhouses, or at chicken foot processing facilities, helps minimize the risk of infection for both poultry and consumers. To date, knowledge of broiler pododermatitis characteristics is limited. Therefore, the objective of this study is to fill this gap by investigating the lesion characteristics of pododermatitis in slaughtered broilers and determining the pathogenic bacteria present in these lesions and their sensitivity to acetic acid.

2. Materials e Methods

a. Ethical approval

No ethical approval was obtained because this study did not involve laboratory animals and only involved non-invasive procedures.

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b. Chicken selection and sampling

This study was conducted in two poultry slaughterhouses in Algeria. The samples were collected from 29 farms distributed across the eastern region of the country. Broilers were reared in buildings equipped with wood chip bedding. All broilers, of mixed sexes, were fed Algerian commercial feed and a standard fattening diet (starter, grower and finisher), and vaccinated according to a standard vaccination protocol. They were slaughtered on the 45th day. A total of 136 broilers with pododermatitis were randomly selected after slaughter. The carcasses were obtained at the *post-mortem* inspection line, and the legs were then examined, swabbed, and photographed using a digital camera. Each sample consisted of a single leg per bird. This procedure made it possible to collect data on the lesional and bacteriological characteristics of these foot lesions.

c. Visual annotation of pododermatitis

Affected legs were visually assessed and classified for pododermatitis by one person, according to a visual scoring system described by Welfare Quality (2009). Based on personal observations, macroscopic scores were assigned to each lesion to assess its severity, using the lesion characteristics reported by Piller et al. (2020) for pododermatitis. The scoring scale has five levels (0, 1, 2, 3, and 4) and is represented by photos (Figure 1). Table 1 shows the lesion characteristics of each pododermatitis score, according to the report by Piller et al. (2020).

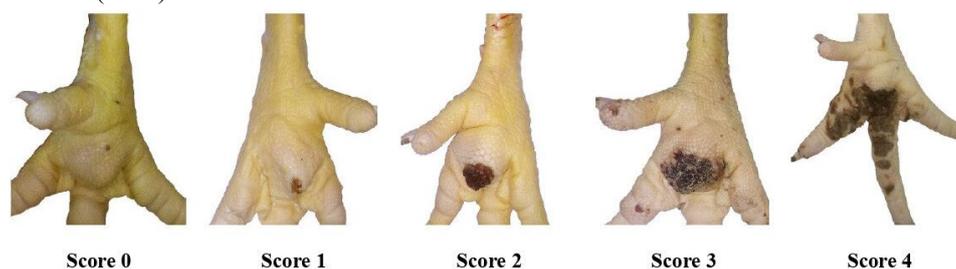


Figure 1 – Visual scoring scale for evaluating pododermatitis, recommended by Welfare Quality (2009).

Score	Lesion characteristics
0	Physiological, non-lesional skin
1	Single superficial* lesion or several cumulative superficial* lesions or deep lesions** ≤ 0.5 cm
2	Superficial* lesion > 0.5 cm or deep** lesion > 0.5 cm to ≤ 1.0 cm
3	Deep** lesion > 1.0 cm
4	Lesion on the footpad and one or more deep lesions** on the toe

*: Lesions with discoloration, hyperkeratosis, no visual disturbance of epidermal layers; **: Lesions with loss of scales, visual disturbance of epidermal layers

Table 1 – Characteristics of the lesions corresponding to each pododermatitis score, according to Piller et al. (2020).

d. Macroscopic measurements of pododermatitis

Photographs of affected legs were used to macroscopically measure the size of each pododermatitis lesion using ImageJ software (*ImageJ 1.54d; Java 1.8.0_345, 64-bit*).

e. Histopathological analysis of pododermatitis

Fifty samples of pododermatitis lesions were collected (10 samples for each score) and placed in vials containing 10% formalin for histopathological analysis. Fixed tissues were dehydrated, cleared, and embedded in paraffin, and 5 µm histological sections were prepared and stained with Hematoxylin and Eosin (H&E) according to standard histological methods. Microscopic examination was performed using an Euromex[®] microscope equipped with a camera and Image Focus Plus V2 software, at different magnifications to detect microscopic lesions in the epidermis and dermis of the foot pad.

f. Bacteriological analysis of pododermatitis

A total of 129 samples of pododermatitis lesions collected from two poultry slaughterhouses were submitted for bacteriological analysis, excluding seven small pododermatitis lesions (score 1) that were not suitable for swabbing. The pododermatitis lesion samples had all different scores. Bacteriological analysis was carried out within 24 h of collection. The swabs were pre-enriched for 24 h at 37 °C in Brain Heart Infusion (BHI) (BIOKAR[®], France). Then, 10 µL of each turbid broth was directly plated onto Chapman agar (BIOKAR[®], France) and Hektoen agar (BIOKAR[®], France), and cultured aerobically at 37 °C for 24-48 h to detect contamination with staphylococci and enterobacteria, respectively. Salmonella detection was carried out according to the ISO 6579-1:2017 method. The different colonies were subcultured on the same culture media for purification, and the bacterial strains were identified using standard microbiological and biochemical tests with API Staph and API 20E test strips (BioMérieux[®], France) (Markey et al., 2013).

g. Determination of the MIC of acetic acid

All bacterial strains were subjected to an agar dilution test to determine the minimum inhibitory concentration (MIC) of acetic acid. This test was performed in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI,

2018). Muller-Hinton agar (BIOKAR[®], France) was used for this protocol, with acetic acid concentrations adjusted to 2.5%, 1.25%, 0.63%, 0.31%, 0.16% and 0.08% (V/V). The agar was then inoculated with a standardized bacterial suspension at a density corresponding to the McFarland 0.5 standard (approximately 10⁸ CFU/mL). This suspension was diluted to 10⁷ CFU/mL, and 2 µL of this dilution was spotted on the agar plates, corresponding to approximately 10⁴ CFU per spot. An agar plate without acetic acid served as a control. After aerobic incubation at 37 °C for 24 h, the plates were visually inspected for bacterial growth. The absence of growth indicated sensitivity to the tested acetic acid concentration, while the presence of bacterial colonies indicated resistance.

3. Results

a. Scoring and morphometry of pododermatitis lesions

During the visual and morphometric evaluation of pododermatitis, it was observed that score 4 was the most frequent (51/136; 37.5%) with an average area of 2.91±2.00 cm², followed by score 2 (42/136; 30.88%) with an average area of 0.5±0.18 cm², score 3 (23/136; 16.91%) with an average area of 1.29±0.79 cm², while score 1 was the least frequent (20/136; 14.71%) with an average area of 0.15±0.08 cm². The rates and areas of each pododermatitis score are shown in Table 2.

Lesion score	Number and rate of affected legs	Lesion area (mean ± standard deviation)
Score 1	20/136 (14.71%)	0.15±0.08 cm ²
Score 2	42/136 (30.88%)	0.5±0.18 cm ²
Score 3	23/136 (16.91%)	1.29±0.79 cm ²
Score 4	51/136 (37.5%)	2.91±2.00 cm ²

Table 2 – Prevalence and morphometry of the different pododermatitis scores.

b. Histopathology of pododermatitis

Concerning histopathological analysis of pododermatitis, score zero corresponded to a healthy skin structure, without histological abnormalities, with regular thickness of the keratin, epidermis, and dermis layers. Score 1 was characterized by mild epidermal hyperkeratosis and hyperplasia, as well as superficial dermal edema, congestion, and mild inflammatory infiltration. Score 2 showed marked hyperkeratosis and hyperplasia of the epidermis, accompanied by areas of inflammation with loss of epidermis in parts of the lesion, as well as superficial dermal congestion and inflammatory infiltration. Score 3 was mainly characterized by ulceration, with full-thickness necrosis of the epidermis replaced by necrotic and suppurative material, as well as marked hyperkeratosis, loss of epidermis, and an area of diffuse inflammation in the dermis. Score 4 corresponded to microscopic lesions that were seen in score 3. Figure 2 presents the different histological characteristics associated with each pododermatitis score in broiler chickens.

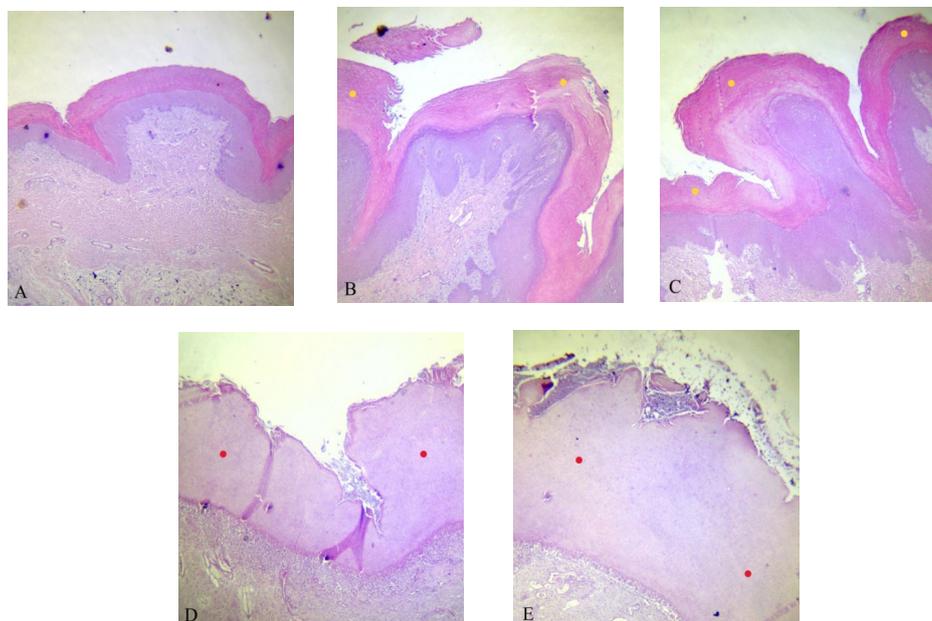


Figure 2 – Histological characteristics of different pododermatitis lesion scores (A: score 0, B: score 1, C: score 2, D: score 3 and E: score 4). Hyperkeratosis (yellow point); necrosis (red point) (H&E staining, 40X).

c. Bacteriology of pododermatitis

All 129 samples taken for bacteriological analysis of pododermatitis were positive, and allowed the isolation of 184 bacterial strains belonging to six different species. Of these samples, the majority (91/129; 70.54%) had infection with a single bacterial

species, while 26/129 samples (20.16%) had co-infection with two bacterial species, 9/129 samples (6.98%) a co-infection with three bacterial species, 2/129 samples (1.55%) a co-infection with four bacterial species, and 1/129 sample (0.77%) a co-infection with five bacterial species. Table 3 presents the number and rate of samples with co-infections.

Number of bacterial species of co-infection	Number and rate of samples
1	91/129 (70.54%)
2	26/129 (20.16%)
3	9/129 (6.98%)
4	2/129 (1.55%)
5	1/129 (0.77%)

Table 3 – Prevalence of bacterial co-infections in pododermatitis lesions.

The isolated bacteria belong to two bacterial families, with a higher rate among staphylococci (133/184; 72.28%) than among enterobacteria (51/184; 27.72%). The most frequently isolated bacterial strain was *Staphylococcus aureus*, representing 122/184 isolates (66.30%), followed by *Escherichia coli*, with 36/184 isolates (19.57%). The other bacterial species, such as *Staphylococcus hyicus*, *Shigella flexneri*, *Shigella sonnei*, and *Salmonella enterica*, were isolated at lower rates, with 11/184 isolates (5.98%), 8/184 isolates (4.35%), 4/184 isolates (2.17%), and 3/184 isolates (1.63%), respectively. Table 4 summarizes the number and rate of bacterial species isolated from 129 pododermatitis lesions.

	Bacteria	Number	Rate (%)
Staphylococci	<i>Staphylococcus aureus</i>	122/184	66.30%
	<i>Staphylococcus hyicus</i>	11/184	5.98%
	Total	133/184	72.28%
Enterobacteria	<i>Escherichia coli</i>	36/184	19.57%
	<i>Salmonella enterica</i>	3/184	1.63%
	<i>Shigella flexneri</i>	8/184	4.35%
	<i>Shigella sonnei</i>	4/184	2.17%
	Total	51/184	27.72%

Table 4 – Prevalence of bacterial species isolated from pododermatitis samples in 129 chickens.

d. MIC of acetic acid

The MIC of acetic acid was evaluated, and all bacterial strains had a MIC \leq 0.08%.

4. Discussion

Pathogenic bacteria associated with pododermatitis have not received much attention. However, these pathogenic bacteria can induce other types of infections in chickens with pododermatitis or be transmitted to consumers of white meat by the food chain (Thøfner et al., 2019). The in-depth study of pododermatitis in broilers and associated bacteria is crucial not only for animal welfare but also for the quality of broiler feet for human consumption. The lesional characteristics of pododermatitis may directly affect the quality of chicken feet as food and as a raw material for gelatin. Skin lesions can alter the texture, appearance, and wholesomeness of chicken feet, thereby influencing their attractiveness in the food market. Furthermore, the presence of bacteria associated with pododermatitis underscores the importance of maintaining strict health standards in broiler foot production to protect public health. By understanding these aspects, researchers can help develop breeding practices and processing procedures that preserve the quality of broiler feet as a food and raw material, thereby ensuring food safety and consumer satisfaction.

According to Hashimoto et al. (2013), there is a positive correlation between the severity of pododermatitis and the condemnation rate, while a negative correlation was observed between this disease and live weight and thigh meat yields. Therefore, controlling pododermatitis could play a vital role in reducing condemnations and improving broiler performance.

In a study conducted by Dinev et al. (2019) in Bulgaria, the prevalence of contact dermatitis lesions in broilers was 21.87%. Pododermatitis was the most common lesion, affecting 15.7% of chickens (Dinev et al., 2019). In another study conducted in Japan on 45 flocks of broilers, all the birds examined had lesions of pododermatitis. The incidence of pododermatitis ranged from 31.9% to 99.5% across flocks, with lesion scores ranging from 0 to 3. Lesion scores affected 13.1, 33.3, 33.4, and 20.2% of broilers, respectively (Hashimoto et al., 2011). The study by Martrenchar et al. (2002) showed that only 10% of broiler flocks were of high quality, with 80% of birds scoring 0 for pododermatitis lesions. In addition, de Jong et al. (2012) reported that in 386 Dutch flocks, 35.5% of broilers showed no lesions, while 26.1% and 38.4% showed mild or severe lesions, respectively, and the severity of pododermatitis decreased with age.

Our study revealed histopathological lesions with different pododermatitis scores, similar to those reported by Piller et al. (2020), thereby corroborating our results. In our study, the scores for lesions 3 and 4 were similar and, apparently, caused by a pathogen-driven inflammatory process, which poses a risk to public health. Furthermore, our results corroborate those of Martins et al. (2016), who reported that higher scores are associated with more severe dermal and epidermal lesions. Additionally, our study showed that increases in the macroscopic score are associated with greater severity of microscopic lesions and deeper inflammation, as also reported by Piller et al. (2020).

The results of our study on the identification of bacteria isolated from pododermatitis agree with those reported by several authors, who also isolated the same bacterial species (*S. aureus*). Youssef et al. (2019) reported that *S. aureus* was isolated from 45.8% of affected chickens, either in pure form (18.18%) or in mixed culture with other bacterial species, including *E. coli* (58.18%), *Proteus mirabilis* (14.67%), and *Pseudomonas aeruginosa* (9.1%). Olsen et al. (2018) identified bacteria from pododermatitis lesions in laying hens, with a predominance of *S. aureus* (68%), followed by *Enterococcus faecalis* (14%), *E. coli* (9%), *S. hyicus* (4.5%), *Gallibacterium anatis* (2.7%), *Trueperella pyogenes* (1.8%), and *Aerococcus urinaeequi* (0.9%). In another study, Chaudhary et al. (2018) found that pododermatitis due to *S. aureus* was present in 89.65% of laying hens, 80.64% of broilers, and 89.28% of backyard poultry.

The results of our study highlight the frequent isolation of *S. aureus*, *E. coli*, and, to a lesser extent, *S. enterica* from pododermatitis lesions in broiler chickens. These bacterial species are known to cause food poisoning in humans. It is therefore essential that veterinary practitioners and poultry slaughterhouse inspectors pay particular attention to this condition and take the necessary precautions to control these pathogenic bacteria and protect public health. Pododermatitis lesions can serve as sources of pathogenic bacteria in poultry slaughterhouses. During the slaughter of chickens, these bacteria can spread through the environment and contaminate chicken meat. These pathogenic bacteria can then cause infections in humans, either through direct contact or by consuming contaminated food or meat.

As for the MIC of acetic acid, it was found to be <0.08% for almost all bacterial strains. These results are encouraging and corroborate previous research. Derbal and Hanfer (2024) also demonstrated that the MIC of acetic acid was 0.08% against isolates of *S. aureus* and *E. coli* from the environment of poultry slaughterhouses, as well as from the carcasses and offal of broiler chickens. Fraise et al. (2013) demonstrated that acetic acid was effective at concentrations as low as 0.166% against various bacterial pathogens. Furthermore, Amrutha et al. (2017) found MICs of 1.5% for *E. coli* and 1% for *Salmonella* sp. Ouattara et al. (1997) also reported that acetic acid concentrations ranging from 0.1% to 1% (W/V) inhibited the growth of several bacteria responsible for meat spoilage. These results confirm that acetic acid is a potent and effective inhibitor of pathogenic bacteria.

Various studies have demonstrated the antibacterial effectiveness of acetic acid in reducing bacterial load on broiler carcasses. These studies have focused on a diverse range of bacteria, including mesophiles, coliforms, *E. coli*, and *S. aureus* (Abdul Wahid, 2008), *C. jejuni* (Zhao and Doyle, 2006), and *S. typhimurium* (Tamblyn and Conner, 1997). Furthermore, Ouattara et al. (1997) demonstrated that acetic acid completely inhibits the growth of bacteria responsible for meat spoilage. Therefore, the application of acetic acid to decontaminate feet affected by pododermatitis could significantly reduce the microbial load in the environment of poultry slaughterhouses, thereby favoring the production of salubrious broiler feet.

Decontamination of feet affected by pododermatitis should help reduce microbial biofilms in a meat processing environment (Ducková et al., 2023). Decontamination of feet affected by pododermatitis should be added as a food safety management choice. Prerequisite programs, such as good hygienic practice (GHP)/good manufacturing practice (GMP)/sanitation standard operating procedure (SSOP), must be established before the implementation of the HACCP (hazard analysis and critical control point) system that more closely controls the risks to human health, as well as the prevention of modifications of foodstuffs by means of control practices in all stages of white meat production (de Oliveira et al., 2016). GHP/GMP/SSOP programs are based on the exclusion and elimination of unwanted and foreign materials, with the inhibition and destruction of pathogenic microorganisms. The integration of GHP/GMP/SSOP programs with the HACCP system ensures process hygiene and impacts on meat safety, thereby helping control foodborne diseases (de Oliveira et al., 2016).

5. Conclusion

In conclusion, according to the results of this study, the highest score (4) was the most common in cases of pododermatitis. Our results revealed the involvement of six bacterial species in pododermatitis in broiler chickens: *S. aureus*, *S. hyicus*, *E. coli*, *S. flexneri*, *S. sonnei*, and *S. enterica*. Staphylococci have been identified as being more common than enterobacteria in pododermatitis lesions, with *S. aureus* being the primary causative agent. A single pododermatitis lesion can be co-infected with up to five bacterial species, although the majority of pododermatitis lesions are infected with a single bacterial species.

The results of this study demonstrate that cleaning/washing feet with acetic acid significantly decreased the prevalence of pathogenic bacteria. It is imperative to establish GMP and SSOP programs, followed by the HACCP system, to help reduce the presence of pathogenic bacteria in the poultry slaughterhouse environment. In turn, the chicken meat produced will carry lower levels of contamination with pathogenic microorganisms. It should therefore be safer from a public health point of view, improving animal welfare.

Conflict of Interest: The authors declare no potential conflicts of interest.

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