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Microbial dynamics of ovine footrot: influence of management systems and lesion type on bacterial community composition

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Summary

The microbial community plays a key role in animal health, influencing immunity, metabolism, and disease resistance. Ovine footrot is a complex bacterial disease involving *Dichelobacter nodosus*, *Fusobacterium necrophorum*, and other members of the hoof microbiome. This study investigated bacteriome dynamics in two sheep management systems – seasonal migration (System A) and permanent indoor housing (System B) – and their association with two clinical forms: interdigital dermatitis (ID) and virulent footrot (VF). A total of 371 bacterial strains were identified from 80 samples collected in many flocks of sheep located in Transylvania. System A showed dominance of Gram-positive bacteria (53.41%), while System B was characterized by a higher prevalence of Gram-negative bacteria (62.86%). ID lesions were predominantly associated with Gram-positive bacteria (65%), whereas VF lesions were largely linked to Gram-negative bacteria (75.4%). These findings illustrate shifts in bacterial landscape during footrot progression and suggest that management practices may influence the composition of disease-associated bacteriota.

Keywords: bacterial flora, sheep, management systems, interdigital dermatitis, virulent footrot

Footrot is a bacterial infection that primarily affects the interdigital skin, leading to a well-defined clinical disease characterized by lameness. This condition poses significant concern for both animal welfare and the economic sustainability of sheep farming worldwide (1). The clinical presentation of footrot can range from mild interdigital dermatitis, which is considered a benign form of the disease, to more severe manifestations, such as heel underrunning, sole and abaxial wall separation, and, in advanced cases, the complete detachment of the hoof capsule, known as virulent footrot (2).

Footrot is a contagious, multifactorial, and polymicrobial hoof disease of ruminants, influenced by different factors, including host susceptibility, farm management practices, environmental conditions, the virulence of *Dichelobacter nodosus*, and the presence of co-infecting bacteria, such as *Fusobacterium necrophorum* (3). *Dichelobacter nodosus*, an anaerobic Gram-negative bacterium, is recognized as the main causative agent of this pathology (3, 4). Besides sheep, other domesticated animals, such as cattle, goats, and South American camelids, as well as wild ruminants, can harbor *D. nodosus*. However, clinical signs are rarely expressed in these species (5). Moreover, *D. nodosus* cannot induce the disease independently, requiring the presence of *Fusobacterium necrophorum*, another anaerobic bacterium. Other bacterial species, such as *Treponema* spp. and *Arcanobacterium pyogenes*, have also been associated with footrot (6, 7). Additionally, microbiota studies, along with the cultivation of aerobic and anaerobic bacteria from both healthy and diseased feet, have revealed the presence of other microorganisms, including *Bacteroides* spp., *Porphyromonas* spp., *Clostridium* spp., *Prevotella* spp., and *Peptostreptococcus* spp. (3, 8).

Moreover, the infection with *Dichelobacter nodosus* appears to induce a shift in the composition of the bacterial community, transitioning from predominantly Gram-positive aerobic taxa in the first period of disease to predominantly Gram-negative, anaerobic taxa as the pathology progresses (7).

This study aimed to investigate the role of bacterial communities in the pathogenesis of ovine footrot by characterizing the hoof microbiota of sheep managed under two contrasting farming systems: seasonal migration and permanent housing. Comparative analysis of these systems was performed to identify differences in microbial composition associated with management practices. Furthermore, the study explored the potential contribution of specific bacterial taxa to disease development and evaluated the relationship between management factors, bacterial diversity, and footrot clinical evolution.

Material and methods

Sample collection. This study was conducted between January and December 2024 in ten Turcana sheep flocks located in Transylvania, Romania, which were managed under two distinct farming systems. The first system followed a seasonal migration pattern known as transhumance, a traditional practice involving the movement of livestock between grazing areas according to seasonal changes. During spring (April-May), shepherds leave their villages and gradually move toward mountain pastures, where they remain throughout the summer, living in temporary shelters. In autumn (September-October), the animals descend from the mountains, and during winter (November-March), sheep graze on plains or in sheltered valleys, depending on precipitation levels. The diet of sheep raised under Romanian transhumance systems varies seasonally, shifting from nutrient-rich alpine pastures in summer to hay-based rations in winter, which are supplemented with cereals. The second system involved permanent housing, with sheep kept indoors throughout the year, with a deep straw bedding on the floor. In this system, animals reside in stable barns and occasionally graze near the farm, with their diet primarily consisting of silage and cereal grains.

The year 2024 in Transylvania was characterized by a strongly above-average thermal regime, with temperatures well above climatological averages, a record heatwave summer, a very mild winter, and unevenly distributed precipitation marked by episodes of atmospheric instability and torrential rainfall.

Within each selected flock, all animals were systematically examined for signs of lameness, with particular focus on any clinical indicators of footrot. Sheep included in the study exhibited lesions corresponding to various stages of footrot progression, which made it possible to perform a comprehensive analysis of bacterial communities associated with the disease. Some animals presented interdigital dermatitis (ID), the initial stage of footrot, characterized by inflammation of the interdigital skin, a pink to reddish discoloration, accumulation of necrotic debris, and a pungent odor. In contrast, more advanced cases exhibited detachment of the hoof horn from the underlying dermal tissues, exudation of a greyish, purulent material, and a strong malodor indicative of necrosis. These clinical signs are consistent with severe bacterial infection, which, if not treated promptly, can lead to pronounced lameness and considerable economic impact. Throughout the study, a total of 80 samples were collected and subjected to microbiological analysis. Samples were collected only from sheep presenting lesions, with animals showing varying degrees of lameness being selected from each flock. Since each sheep had lesions on only one limb, a single sample was collected per animal, thus avoiding pseudoreplication of the bacterial strains isolated at this level. To ensure a representative approach, half of the samples (n = 40) were collected from sheep raised under a seasonal migration system, while the remaining 40 samples were collected from sheep housed in a permanent indoor system.

Tissue samples were collected from the interdigital space of sheep affected by footrot, with one sample taken per affected foot. Sampling was performed using Amies transport medium swabs (HiMedia Laboratories, India), with charcoal for anaerobic bacteria and without charcoal for aerobic organisms. Swabs were carefully rotated within the interdigital space and, when applicable, inserted into deeper hoof lesions. After collection, samples were transported to the laboratory under appropriate biosafety conditions. Microbiological processing was initiated within 4 to 10 hours post-collection.

Isolation of anaerobes. Anaerobic bacteria were initially inoculated in liquid medium by suspending each swab in a tube containing a thioglycolate broth (HiMedia Laboratories, Germany), which supports bacterial growth under reduced oxygen conditions. An essential consideration in cultivating anaerobic bacteria in broth cultures is the choice of culture tubes, as parameters such as oxygen permeability, tube material, and cap design significantly influence anaerobic conditions (9). To ensure strict anaerobic conditions, Hungate tubes (Bellco Biotechnology, USA), specifically designed to minimize oxygen exposure and maintain strict anaerobic conditions, were employed in this study. Following inoculation, the tubes were incubated at 37°C for 48-72 hours. Secondary cultures were performed on solid media, specifically Brucella blood agar (BioMaxima S.A., Lublin, Poland). Each plate was inoculated using material from the enriched thioglycolate cultures. The selection of appropriate culture media is critical to ensure the successful isolation and growth of clinically relevant anaerobic bacteria. Brucella blood agar was selected for its broad-spectrum capability as an enriched, non-selective medium that supports the growth of most clinically significant anaerobic species (10, 11). After the initial incubation, colonies were visually inspected and selected based on their morphological characteristics, including color, size, margin, elevation, and hemolysis pattern. For each sample, one representative colony per morphotype was selected to capture bacterial diversity while avoiding duplication of bacterial taxa. Selected colonies were subcultured to obtain pure isolates prior to identification with MALDI-TOF MS or the API 20A system. Sub-cultured plates were incubated anaerobically at 37°C for a minimum of 48 hours. Anaerobic conditions were achieved using an anaerobic jar equipped with the AnaeroCult® system (Merck KGaA Darmstadt, Germany) (12), which effectively removes oxygen while simultaneously generating carbon dioxide to create an optimal environment for anaerobic bacterial growth. Before the application of definitive identification techniques, the colonies were subjected to a series of preliminary evalu-

ations, including macroscopic colony morphology, Gram staining, catalase activity, fluorescence under UV light, and an aerotolerance test.

Identification of anaerobic bacteria with API 20A system and MALDI-TOF MS. Identification of the anaerobic bacterial strains isolated from clinical samples was performed using the commercially available API® 20A system (bioMérieux, France), a standardized miniaturized test kit specifically designed for the biochemical characterization of anaerobes. Bacterial isolates were suspended in API® 20A inoculation medium to a turbidity equivalent to the 3.0 McFarland standard. The suspension was then used to rehydrate the dehydrated substrates present in each of the 20 microtubes of the API® 20A strip. The test strips were incubated under strictly anaerobic conditions at 37°C for 24 hours. In this system, metabolic activity of the bacteria induces colorimetric changes in the media, which occur either spontaneously or after the addition of specific reagents (e.g., BCP, XYL, HER). While most anaerobic species yield interpretable biochemical profiles within 24 hours, reliable identification of certain slow-growing strains requires extended incubation for up to 48 hours at 37°C for.

Following incubation, the biochemical reactions were assessed visually and interpreted using the API Reading Table. Final bacterial identification was conducted using the bioMérieux identification software. In addition to the 20 biochemical tests, three morphological and staining parameters – spore formation (SPOR: +/−), Gram stain reaction (GRAM: +/−), and cellular morphology (coccus shape, COCC: +/−) – were also recorded and included in the final identification profile.

The MALDI Biotyper® Sirius System (Bruker, Ettlingen, Germany) identifies bacterial strains using mass spectrometry by matching the acquired spectra with reference spectra stored in its database. The analysis was performed in accordance with the manufacturer's instructions.

Identification of aerobic bacteria. Each specimen was initially inoculated into nutrient broth to evaluate the presence of aerobic bacteria in footrot lesion samples (Oxoid, UK). The inoculated broth was incubated at 37°C for 24 hours under aerobic conditions to promote the growth of oxygen-dependent bacterial species. Following this enrichment step, the cultures were streaked onto nutrient agar plates using sterile inoculating loops to isolate individual bacterial colonies. The inoculated agar plates were incubated aerobically at 37°C for 24-48 hours. After incubation, colonies exhibiting distinct morphological characteristics were selected and subcultured onto fresh nutrient agar plates using the same aseptic techniques. This step was critical to obtaining pure cultures, ensuring that each isolate represented a single bacterial strain. Pure colonies were subsequently subjected to biochemical identification procedures to determine the species involved in the aerobic component of the footrot lesions.

Following primary isolation, bacterial identification was performed using appropriate biochemical profiling systems, including API20E, API50CHB, APIStaph, or APIStrepto (BioMérieux SA, Marcy-l'Étoile, France), selected based on the morphological and staining characteristics of each isolate. Macroscopic and microscopic examinations were first conducted to assess colony morphology, Gram reaction, and cellular arrangements. In addition, catalase and oxidase tests were employed as preliminary screening tools to assist in genus-level differentiation and guide the selection of appropriate API systems.

API system strips were prepared and inoculated following the manufacturer's instructions. After incubation at 37°C for 24 hours, biochemical reactions were recorded and interpreted using the APIWEB identification software (bioMérieux, France), an online platform that compares phenotypic profiles to an extensive reference database to predict species identity (13).

Statistical analysis. Data were compiled using Microsoft Office Excel 2021 and statistically analyzed with GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Descriptive statistics were used to illustrate the proportions of various bacterial findings in the two management systems. Fisher's exact test analyzed the association between lesion stage (interdigital dermatitis and virulent footrot) and bacterial presence at the flock level.

Results and discussion

In this study, 371 bacterial species (n = 371) were identified in association with various pathological conditions. Of these, 161 bacterial strains (n = 161) were detected in samples obtained from sheep raised under a seasonal migration system (System A), whereas 210 bacterial strains (n = 210) were identified in samples collected from the feet of sheep housed permanently indoors (System B). The results of Fisher's exact test performed to examine the association between the bacterial type (Gram-positive vs. Gram-negative) and the system used (System A vs. System B) revealed a statistically significant association between the two variables ($p = 0.0008$), indicating that the distribution of Gram-positive and Gram-negative bacteria differed significantly between the two systems.

In System A, Gram-positive bacteria were more prevalent (53.41%) than they were in System B (37.14%). Conversely, System B showed a considerably higher proportion of Gram-negative bacteria (62.86%) compared to System A (46.58%) (Fig. 1).

In cases of interdigital dermatitis, the bacterial population was predominantly composed of Gram-

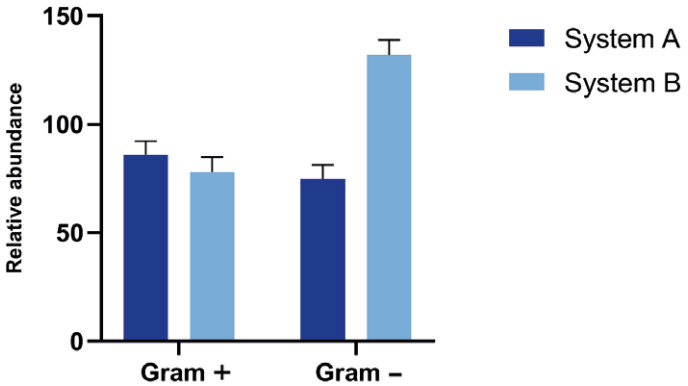

Fig. 1. Distribution patterns of Gram-positive and Gram-negative bacterial isolates stratified by husbandry system. Error bars represent the standard error of the mean

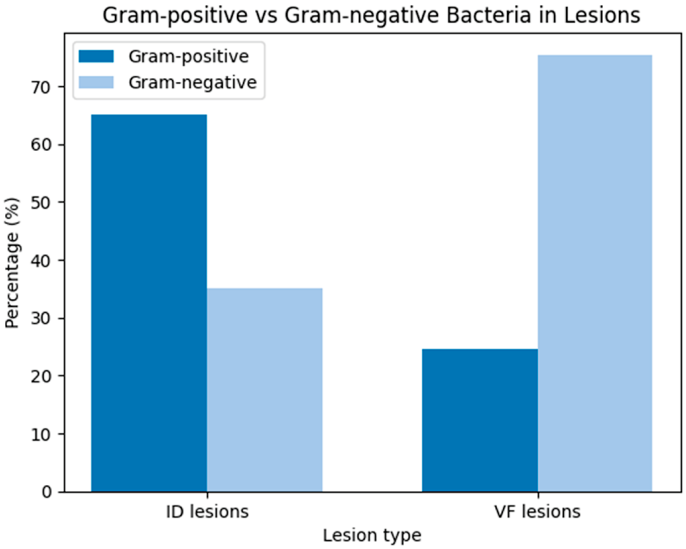

Fig. 2. Distribution patterns of Gram-positive and Gram-negative bacterial isolates stratified by lesion stage

positive bacteria, while Gram-negative bacteria were more frequently associated with virulent footrot, the second stage of the disease. Among the 180 bacterial isolates from ID lesions, 65% were Gram-positive and 35% were Gram-negative. In contrast, of the 191 bacterial isolates from VF lesions, 75.40% were Gram-negative and 24.60% were Gram-positive (Fig. 2). The results of Fisher's exact test performed to evaluate the association between bacterial Gram type and lesion type in ovine foot disease demonstrated a statistically significant relationship between these variables ($P < 0.0001$), indicating that the distribution of Gram-positive and Gram-negative bacteria differed significantly between interdigital dermatitis (ID) and virulent footrot (VF). This pattern suggests a predominance of Gram-positive bacteria in ID lesions and a clear shift toward Gram-negative bacteria in VF.

The composition of the bacterial community in samples taken from sheep raised in the seasonal migration system (System A). A total of 161 bacterial strains were identified from samples collected from sheep reared under a seasonal migration system (n = 40). Table 1 summarizes the distribution of these bacterial strains in the two stages of pathology observed in this system.

Aerobic bacterial strains were classified into six families (Enterobacteriaceae, Pseudomonaceae, Bacillaceae, Streptococcaceae, Micrococcaceae, and Staphylococcaceae). Among these, the most dominant family was Enterobacteriaceae, accounting for 25.46% (n = 41) of all strains identified. This family comprised four distinct genera: *Escherichia*, *Enterobacter*, *Klebsiella*, and *Serratia*. The second most representative bacterial family identified was Bacillaceae, accounting for 16.14% of all bacteria detected. This family consists of Gram-positive, rod-shaped, spore-forming bacteria known for their resilience in various environmental conditions (14). Within the Bacillaceae family, the only identified genus was *Bacillus*, with 26 strains of *Bacillus subtilis* detected in the samples. Other aerobic bacterial strains identified in the samples included *Pseudomonas aeruginosa*, *Micrococcus* spp., *Streptococcus* spp., and *Staphylococcus* spp. *Pseudomonas aeruginosa* accounted for 15.52% (n = 25) and *Micrococcus* spp. for 9.31% (n = 15) of the total bacteriota identified. *Streptococcus* spp. was represented by two species, *Streptococcus uberis* and *Streptococcus intermedius*, each identified in 5 samples collected from the feet of sheep. Additionally, at the lesional level, bacteria from the *Staphylococcus* genus were identified. Among them, *Staphylococcus aureus*, *Staphylococcus sciuri*, and *Staphylococcus simulans* collectively accounted for 6.2% of the total bacterial flora.

The anaerobic bacteriota identified in the samples included *Dichelobacter nodosus* and *Fusobacterium necrophorum*, each comprising 1.24% of the total bacterial population. These two bacterial species are recognized as the primary pathogens responsible for footrot lesions in sheep (4, 15). However, although characteristic footrot lesions were present, successful isolation of these bacteria was not always possible. Specifically, only 2 strains of *Fusobacterium necrophorum* and 2 strains of *Dichelobacter nodosus* were successfully isolated across all samples. Among the anaerobic isolates recovered from foot lesion samples, the most prevalent species was *Prevotella intermedia*, accounting for 44.11% (n = 15) of the total anaerobic

Tab. 1. Stage-specific bacterial profiling of footrot lesions in sheep raised in the seasonal migration system

Isolate	%	Interdigital dermatitis	Virulent footrot
Escherichia coli	11.18 (n = 18)	8	10
Klebsiella pneumonie	3.10 (n = 5)	2	3
Serratia spp.	4.96 (n = 8)	5	3
Enterobacter cloacae	6.21 (n = 10)	3	7
Pseudomonas aeruginosa	15.52 (n = 25)	5	20
Bacillus subtilis	16.14 (n = 26)	18	8
Micrococcus spp.	9.31 (n = 15)	10	5
Streptococcus uberis	3.10 (n = 5)	5	0
Streptococcus intermedius	3.10 (n = 5)	5	0
Staphylococcus aureus	3.10 (n = 5)	5	0
Staphylococcus sciuri	1.86 (n = 3)	3	0
Staphylococcus simulans	1.24 (n = 2)	2	0
Dichelobacter nodosus	1.24 (n = 2)	1	1
Fusobacterium necrophorum	1.24 (n = 2)	0	2
Peptostreptococcus spp.	9.31 (n = 15)	10	5
Prevotella intermedius	9.31 (n = 15)	3	12
Total	n = 161	85	76

flora. An equal number of isolates (n = 15) were identified as *Peptostreptococcus* spp. Thus, out of the 34 anaerobic strains identified, 30 were these two taxa, indicating their potential significance in the microbial landscape of footrot lesions. All bacterial species in the two lesion stages are represented in Figure 3. In ID lesions, the most prevalent bacterial genera were *Bacillus subtilis* (n = 18), followed by *Micrococcus* spp. (n = 10) and *Peptostreptococcus* spp. (n = 10). In VF lesions, the most frequently identified bacterial strains were *Pseudomonas aeruginosa* (n = 20), followed by *Prevotella intermedius* (n = 12).

Statistically non-significant differences were identified in both the isolation rates and bacterial population variability between interdigital dermatitis (ID) and virulent footrot (VF) lesions. The unpaired t-test yielded a P value of 0.7551 (t = 0.3148, df = 30), indicating that the mean difference between the two groups (–0.5625 ± 1.787) was non-significant. The 95% confidence interval for the difference ranged from –4.212 to 3.087, which includes zero, further supporting the absence of a true effect.

Additionally, the F-test for equality of variances resulted in a P value of 0.3878 (F = 1.577, df = 15, 15), showing no significant difference in variance between the groups. This suggests that the spread or dispersion of bacterial values was comparable in both lesion types. Together, these results indicate that the central tendency and variability of the bacterial presence are statistically similar between ID and VF lesions in the conditions analyzed.

The composition of the bacterial community in samples collected from sheep housed permanently indoors (System B). The bacteriological analysis of 40 samples collected from sheep housed permanently indoors on litter identified 210 (n = 210) bacterial strains (Tab. 2).

The most representative bacteria with potential pathogenicity identified in the samples belonged to the Enterobacteriaceae family, with a total of 80 strains detected. This family included four different genera: *Aeromonas*, represented by two species: *Aeromonas hydrophila/caviae* and *Aeromonas veronii*, *Escherichia*, with *Escherichia coli* species, *Enterobacter*, and *Klebsiella*. The species identified from the Enterobacteriaceae family accounted for 38.09% of all bacteria detected in the samples collected from sheep housed indoors. The second most frequently isolated Gram-negative bacterial genus was *Pseudomonas*, represented by two species: *Shewanella putrefaciens* and *Pseudomonas aeruginosa*. Together, these species accounted for 10.33% (n = 25) of all bacteriota identified from the hoof samples. Another

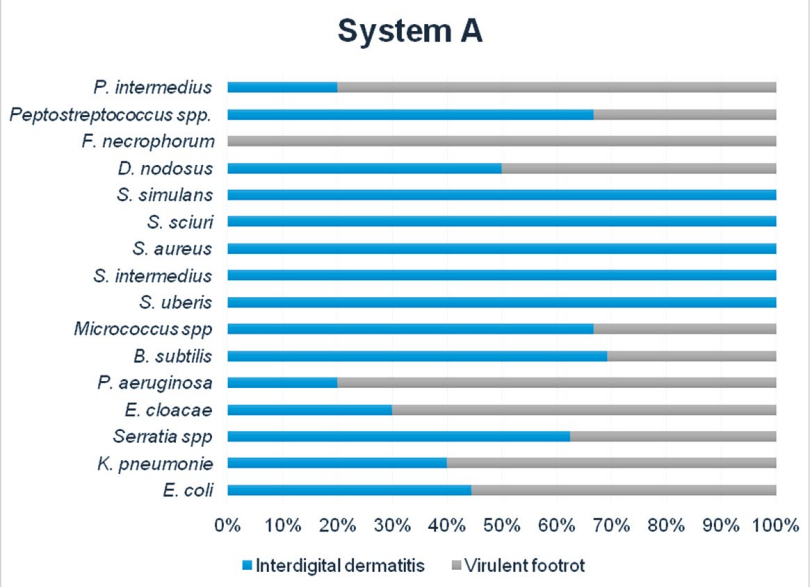

Fig. 3. Relative abundance of bacterial genera and species in the microflora of hoof samples from sheep maintained under system A, stratified by lesion stage. The left bar shows the proportion of bacteria associated with interdigital dermatitis, whereas the right bar shows the proportion of bacteria associated with virulent footrot

Tab. 2. Stage-specific bacterial profiling of footrot lesions in permanently housed sheep

Isolate	%	Interdigital dermatitis	Virulent footrot
Escherichia coli	11.90 (n = 25)	10	15
Aeromonas hydrophila/caviae	4.76 (n = 10)	5	5
Aeromonas veronii	5.57 (n = 15)	5	10
Enterobacter cloacae	5.57 (n = 15)	5	10
Klebsiella pneumoniae	5.57 (n = 15)	5	10
Shewanella putrefasciens	5.57 (n = 15)	5	10
Pseudomonas aeruginosa	4.76 (n = 10)	2	8
Acinetobacter iwoffi	4.76 (n = 10)	0	10
Acinetobacter radioresistant	4.76 (n = 10)	0	10
Enterococcus faecium	4.76 (n = 10)	8	2
Kocuria rosea	8.09 (n = 17)	12	5
Micrococcus spp.	4.76 (n = 10)	10	0
Rothia mucilaginosa	0.95 (n = 2)	2	0
Staphylococcus sciuri	3.80 (n = 8)	5	3
Streptococcus uberis	1.42 (n = 3)	3	0
Bacillus cereus	2.38 (n = 5)	5	0
Aerococcus spp.	1.42 (n = 3)	3	0
Dichelobacter nodosus	1.90 (n = 4)	2	2
Fusobacterium necrophorum	2.38 (n = 5)	2	3
Prevotella spp.	6.19 (n = 13)	3	10
Peptostreptococcus spp.	2.38 (n = 5)	3	2
Total	n = 210	95	115

Gram-negative bacterial genus identified in the samples was *Acinetobacter*, comprising two species: *Acinetobacter iwoffi* and *Acinetobacter radioresistens*, with 10 strains of each species isolated. *Enterococcus faecium* was also identified in 10 samples collected from hoof lesions. *Staphylococcus sciuri* is another Gram-positive bacterium isolated from the samples collected from footrot lesions, with 8 strains identified at this level. Other Gram-positive taxa belonging to the Bacillaceae, Streptococcaceae, and Aerococcaceae families were also detected, but their prevalence within the total bacterial population was comparatively low. Specifically, five isolates were attributed to the Bacillaceae family, while Streptococcaceae and Aerococcaceae were each represented by three strains.

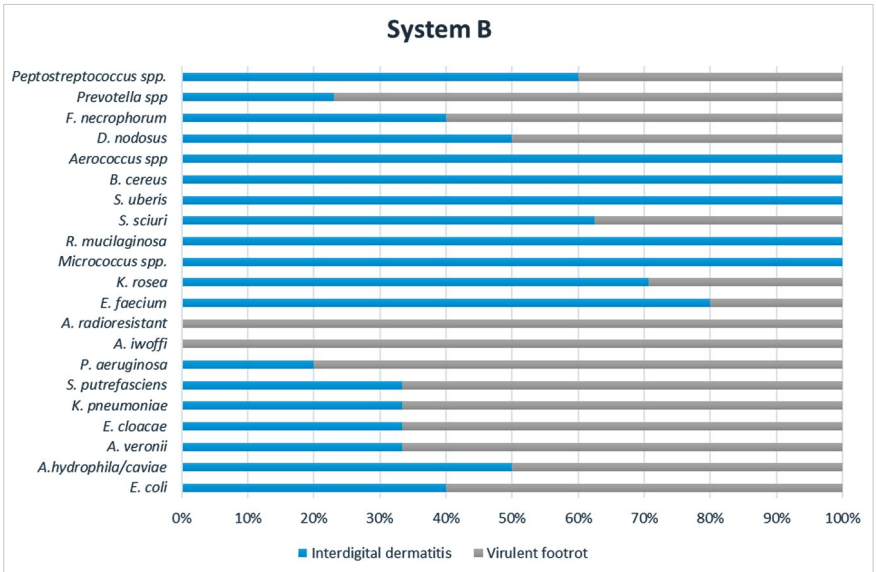

Fig. 4. Relative abundance of bacterial genera and species in the microflora of hoof samples from sheep maintained under system B, stratified by lesion stage. The left bar shows the proportion of bacteria associated with interdigital dermatitis, whereas the right bar shows the proportion of bacteria associated with virulent footrot

Anaerobic bacteria constituted 12.85% of the bacteriota recovered from the hoof samples. The anaerobic components of the bacterial community included *Dichelobacter nodosus*, *Fusobacterium necrophorum*, *Prevotella* spp., and *Peptostreptococcus* spp. The most frequently isolated anaerobic genus was *Prevotella*, comprising 48.14% (n = 13) of the anaerobic isolates. *Fusobacterium necrophorum* was detected in five samples, while *Dichelobacter nodosus* was identified in four. Additionally, *Peptostreptococcus* spp., a Gram-positive anaerobe, was isolated from five samples. Figure 4 shows the distribution of all bacterial types across the lesion stages. In ID lesions, the most prevalent bacterial genera were *Kocuria rosea* (n = 12), followed by *Micrococcus* spp. (n = 10) and *Escherichia coli* (n = 10). In VF lesions, *E. coli* was the most frequent (n = 15), followed by *Aeromonas veronii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Shewanella putrefaciens*, *Acinetobacter iwoffi*, *Acinetobacter radioresistant,* and *Prevotella* spp., each represented by 10 strains.

The unpaired t-test performed to compare the bacterial values between interdigital dermatitis (ID) and virulent footrot (VF) lesions showed that the isolation rate was 5.476 for VF and 4.524 for ID. Although VF showed a slightly higher mean, the difference between the two groups (0.9524 ± 1.239) was statistically non-significant ($t = 0.7688$, $df = 40$, $P = 0.4465$). The 95% confidence interval for the difference (–1.551 to 3.456) includes zero, indicating no reliable difference between lesion types.

Additionally, the F-test for comparing variances yielded a value of $F = 2.140$ with $P = 0.0967$, suggesting that although there was a numerical difference in variances, this was statistically non-significant at a confidence level of 0.05. The effect size ($R^2 = 0.01456$) indicates a very small effect, meaning that the lesion type accounted for only about 1.5% of the variance in bacterial values.

The findings indicate that both systems create environmental conditions conducive to the development of lesions characteristic of footrot and support the proliferation and virulence expression of associated bacterial populations. Animal hooves are exposed to a wide spectrum of microorganisms, including commensal Gram-positive bacteria, such as *Streptococcus* spp., *Staphylococcus* spp., *Bacillus* spp., and *Micrococcus* spp. In addition, Gram-negative bacteria of fecal origin, such as *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and other *Enterococcus* species, are frequently present in the hoof environment. Opportunistic residents of the hoof capsule, such as *Acinetobacter* spp., can further contribute to the bacterial diversity (16). The pathogenic potential of this bacterial community is strongly influenced by environmental conditions, including hygiene, ambient temperature, precipitation level, high humidity, as well as the structural integrity of the interdigital epithelium (1). This study characterized the bacterial communities associated with footrot lesions in sheep raised under two distinct husbandry systems: seasonal migration and permanent indoor housing with bedding. The bacterial communities identified on the feet of sheep housed permanently indoors exhibited greater taxonomic diversity compared to those associated with sheep raised under a seasonal migration system. Notably, disease progression was accompanied by a marked shift in bacterial composition – from a predominance of Gram-positive bacteria in ID (the early stage of footrot) to a dominance of Gram-negative taxa in under-running lesions, which characterize the more advanced and virulent stage of

the disease. This transition mirrors microbial succession patterns observed in other polymicrobial infections across both human and veterinary contexts, where disease onset is frequently associated with increased bacterial diversity and compositional shifts. Such patterns have been documented in various host-pathogen systems and are thought to reflect a generalized response to the breakdown of homeostasis at the affected body site (7, 17-19).

In this study, the majority of bacterial strains identified belonged to the Enterobacteriaceae family, representing 25.46% of all bacteriota in the samples from sheep raised under the seasonal migration system and 38.09% in the samples from sheep housed permanently indoors. The most prevalent bacterial species identified was *Escherichia coli*, a Gram-negative, rod-shaped bacterium commonly found in the lower intestinal tract of warm-blooded animals and typically excreted into the environment with feces (20). Potentially pathogenic *E. coli* strains are capable of surviving and proliferating in various environments, where their growth is influenced by both biotic factors, such as microbial interactions, nutrient availability, and biofilm formation, and abiotic factors, including temperature, water availability, pH, and solar radiation (21). The high isolation rate of this bacterium under both systems indicated the occurrence of fecal contamination, poor hygiene conditions, and high humidity, essential conditions involved in the pathogenicity of tissue destruction. The role of *E. coli* in the pathogenesis of footrot remains uncertain, but its presence has been documented in hoof lesions of calves, suggesting a potential involvement in tissue damage and secondary infections (22).

Another pathogen from the Enterobacteriaceae family often identified was *Klebsiella* spp., an opportunistic pathogen that can be found in the environment (23, 24), in the gastrointestinal tract of animals, and on the skin of healthy individuals as a benign colonizer (25). In this research, a high proportion of *Klebsiella* strains were isolated from samples collected from sheep raised under seasonal migration. However, certain strains of this genus were also isolated from sheep kept permanently indoors. To date, there are no reports demonstrating the pathogenicity of *Klebsiella* species in the interdigital skin of ruminants. In the dairy industry, *Klebsiella pneumoniae* – one of the most common causes of mastitis – has been the subject of numerous studies (26-28). The detection of this bacterium in footrot lesion samples is concerning, as *K. pneumoniae* is recognized as a major reservoir of resistance genes, facilitating their transfer from environmental sources to clinically significant bacteria. Additionally, some isolates may harbor acquired antimicrobial resistance (AMR) genes or plasmids, enabling their dissemination across environmental, human, and animal matrices (29). Alongside this pathogen, other bacterial species, including *Enterococcus faecium*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. (30, 31) have also been isolated from footrot lesions. These organisms belong to the ESKAPE group, a cluster of pathogens known for their ability to evade antimicrobial treatments and frequently exhibit a multidrug-resistant (MDR) phenotype (30, 31).

Animal skin is not a sterile environment since it harbors a diverse array of commensal microorganisms (32). *P. aeruginosa*, a ubiquitous bacterium commonly found as part of the normal skin microflora, can become an opportunistic pathogen when the integrity of the skin barrier is disrupted, potentially leading to infections (33). *P. aeruginosa* is primarily an environmental organism, and according to Al-Tememe and Abbas's study (34), more strains of this genus were isolated from environmental samples when compared to those taken from healthy sheep. In the present study, a significant number of *P. aeruginosa* strains were isolated from the hooves of sheep in both raising systems, with a notably higher prevalence in under-running lesions. This finding underscores the opportunistic nature of *P. aeruginosa*, which is capable of colonizing compromised interdigital skin and exploiting tissue damage to establish infection. Another member of the *Pseudomonas* genus identified at the hoof level was *P. putrefaciens*, a bacterium that, based on phylogenetic studies, was reclassified in 1985 to the genus *Shewanella* (35). *Shewanella putrefaciens* is a natural component of marine microflora and is commonly found in moderate and warm climates, where it thrives in aquatic environments rich in organic matter and is rarely pathogenic (36). The pathogenicity of *Shewanella* species remains unclear, partly because they are often found in polymicrobial infections. Nevertheless, accumulating evidence indicates that some species are pathogenic for humans or animals (37). In the veterinary context, this bacterium has been described primarily in studies focusing on its isolation from various food sources rather than as a direct pathogen in animals (38). In contrast, in human medicine, *S. putrefaciens* has been associated predominantly with skin and soft tissue infections (36).

Another Gram-negative bacterium isolated exclusively from sheep housed permanently indoors was the *Aeromonas* genus, commonly found in freshwater, sewage, and soil (39). The presence of *Aeromonas* in the feces of horses, cattle, and sheep was reported in several studies (39), and it has long been recognized as a pathogen of amphibians and reptiles (40).

Among the Gram-positive bacteria, the Bacillaceae family was the most predominant in samples from sheep raised under the seasonal migration system, while the Micrococcaceae family emerged as the dominant group in sheep from the permanent housing system. These bacterial species are common inhabitants of the skin of both humans and animals, and may sometimes produce proteolytic and collagenolytic enzymes that disintegrate the skin (41).

Bacillus species are aerobic, spore-forming, rod-shaped bacteria that are widely distributed in nature (42). They have been isolated from both the interdigital space of healthy cattle and lesions associated with bovine interdigital dermatitis (43). Similarly, they have been found on the interdigital skin of sheep (44) and in horses' hooves (16).

Micrococcus is a bacterial genus that forms part of the normal microbial community residing on the skin and external surfaces of animals. In studies focused on characterizing skin microflora, Gram-positive coccoid bacteria from the Micrococcaceae family were identified as the most prevalent microorganism in sheep flocks from various regions (45). Under certain conditions, depending on ecological, medical, host-related, and microbial factors, these commensal microorganisms can frequently act as opportunistic pathogens and contribute to the development of various diseases (46). Consequently, these bacteria can be classified as minor pathogens in the context of this disease. *Enterococcus faecium*, another Gram-positive bacterium, was isolated exclusively from the bacterial population of sheep hooves in System B. These bacteria are likely a part of the normal hoof flora and typically do not pose any significant risks under healthy environmental conditions. However, they may proliferate when the hoof becomes weakened or infected (47). Several studies have reported the involvement of *E. faecium* in the hooves of horses (47) and cattle with ID (17, 19).

Dichelobacter nodosus is the primary microbial agent responsible for causing footrot in small ruminants (3) and is often found in association with *Fusobacterium necrophorum*, another Gram-negative bacterium frequently isolated from footrot lesions (3, 15). *F. necrophorum*, a normal inhabitant of the animal alimentary tract (48), is also commonly detected in oral (49) and fecal material (50) and has been implicated in the development of foot abscesses in sheep (5, 51). Although these anaerobic bacteria were not detected in every sample, their absence in culture does not rule out their presence at the lesion site, as they may not have had the optimal conditions for growth in the laboratory environment. *D. nodosus* was isolated from 4 out of 50 samples in System B and from 2 out of 50 samples in System A. *F. necrophorum* strains were isolated predominantly from underrunning lesions, which are associated with the more advanced stages of the disease. These findings show that an increased load of *D. nodosus* plays a key role in the pathogenesis of footrot, while *Fusobacterium necrophorum* may act as a secondary invader. While these observations are consistent with previous reports suggesting that *D. nodosus* initiates the infection and *F. necrophorum* contributes to lesion progression, the low number of isolates in this study limits the strength of such inferences. Therefore, these results should be interpreted cautiously and viewed as indicative of possible associations rather than definitive pathogen roles. This interpretation aligns with prior studies showing *D. nodosus* to be more abundant in feet affected by ID than in more advanced footrot (8, 52).

This study offers novel insights into bacterial communities associated with the sheep hoof and constitutes a comparative analysis of microbial distribution across two distinct management systems, stratified by lesion stage. Statistical analysis revealed a significant variation in the relative abundance of Gram-positive and Gram-negative bacteria between the two systems. Specifically, the seasonal migration system was characterized by a predominance of Gram-positive bacteria, whereas the permanent indoor housing system exhibited a higher prevalence of Gram-negative organisms. Concerning the lesion type, Gram-positive bacteria were more frequently isolated from interdigital dermatitis lesions, while Gram-negative bacteria were associated predominantly with virulent footrot cases.

Nonetheless, comparative analysis of the relative abundance of bacterial taxa in interdigital dermatitis and virulent footrot lesions – regardless of the management system – revealed no statistically significant differences in either mean abundance or variability. The composition and abundance of the bacterial community exhibited variability across different flocks, a variation likely influenced by the management system in place or potentially determined by the disease stage.

These findings suggest that the management system may influence the relative abundance of Gram-positive and Gram-negative bacteria. The statistically significant findings (low p-value) indicate an association between the type of system and observable differences in the bacterial profile, potentially reflecting the influence of housing conditions on the bacterial community structure and their implications for animal health.

References

1. *Zanolari P., Dürr S., Jores J., Steiner A., Kuhnert P.*: Ovine footrot: A review of current knowledge. Vet. J. 2021, 271, 105647, doi: 10.1016/j.tvjl.2021.105647, PMID: 33840488.
2. *Abbott K. A., Egerton J. R.*: Effect of climatic region on the clinical expression of ovine footrot. Aust. Vet. J. 2003, 81 (12), 756-762, doi: 10.1111/j.1751-0813.2003.tb14609.x.
3. *Beveridge W. I. B.*: Foot-rot in sheep: a transmissible disease due to infection with Fusiformis nodosus (n. sp.). Studies on its cause, epidemiology, and control. Melbourne: Council for Scientific and Industrial Research 1941, Bulletin No. 140.
4. *Stewart D. J., Claxton P. D.*: Ovine footrot. Aust. Adv. Vet. Sci. 1979, 45-46.
5. *Zhou H., Bennett G., Hickford J. G. H.*: Variation in Fusobacterium necrophorum strains present on the hooves of footrot infected sheep, goats and cattle. Vet. Microbiol. 2009, 135 (3-4), 363-367, doi: 10.1016/j.vetmic.2008.09.084, PMID: 19019570.
6. *Calvo-Bado L. A., Oakley B. B., Dowd S. E., Green L. E., Medley G. F., Ul-Hassan A., et al.*: Ovine pedomics: the first study of the ovine foot 16S rRNA-based microbiome. ISME J. 2011, 5 (9), 1426-1437, doi: 10.1038/ismej.2011.25, PMID: 21430786.
7. *Maboni G., Blanchard A., Frosth S., Stewart C., Emes R., Tötemeyer S.*: A distinct bacterial dysbiosis associated skin inflammation in ovine footrot. Sci. Rep. 2017, 7, 45220, doi: 10.1038/srep45220, PMID: 28338081.
8. *Moore L. J., Wassink G. J., Green L. E., Grogono-Thomas R.*: The detection and characterisation of Dichelobacter nodosus from cases of ovine footrot in England and Wales. Vet. Microbiol. 2005, 108 (1-2), 57-67, doi: 10.1016/j.vetmic.2005.01.029, PMID: 15917134.
9. *Bacic M. K., Smith C. J.*: Laboratory maintenance and cultivation of Bacteroides species. Curr. Protoc. Microbiol. 2008, Chapter 13, Unit 13C.1, doi: 10.1002/9780471729259.mc13c01s9, PMID: 18770533.

10. *Mangels J. I., Douglas B. P.*: Comparison of four commercial Brucella agar media for growth of anaerobic organisms. J. Clin. Microbiol. 1989, 27, (10), 2268-2270, PMID: 2584379.
11. *Hastey C. J., Boyd H., Schuetz A. N., Anderson K., Citron D. M., Dzink-Fox J., et al.*: Changes in the antibiotic susceptibility of anaerobic bacteria from 2007-2009 to 2010-2012 based on the CLSI methodology. Anaerobe 2016, 42, 27-30, doi: 10.1016/j.anaerobe.2016.07.003, PMID: 27427465.
12. *Imhof A., Heinzer I.*: Continuous monitoring of oxygen concentrations in several systems for cultivation of anaerobic bacteria. J. Clin. Microbiol. 1996, 34 (7), 1646-1651, PMID: 8784353.
13. *Lengkey H. A. W., Balia R. L., Togoe I., Taşbac B. A., Ludong M.*: Isolation and identification of lactic acid bacteria from raw poultry meat. Biotechnol. Anim. Husb. 2009, 25 (5-6), 1071-1077.
14. *Zhao P., Xue Y., Gao W., Li J., Zu X., Fu D., et al.*: Bacillaceae-derived peptide antibiotics since 2000. Peptides 2018, 101, 10-16, doi: 10.1016/j.peptides.2017.12.018, PMID: 29269072.
15. *Egerton J. R., Roberts D. S., Parsonson I. M.*: The aetiology and pathogenesis of ovine footrot. I. A histological study of the bacterial invasion. J. Comp. Pathol. 1969, 79 (2), 207-216. doi: 10.1016/0021-9975(69)90008-0.
16. *Kim B. J., Cho G. J.*: Characterization of the microbial flora isolated from horses with hoof thrush and their sensitivity for selected antibiotics. Int. J. Vet. Sci. 2023, 12 (4), 522-527, doi: 10.47278/journal.ijvs/2023.005.
17. *Krull A. C., Shearer J. K., Gorden P. J., Cooper V. L., Phillips G. J., Plummer P. J.*: Deep sequencing analysis reveals temporal microbiota changes associated with development of bovine digital dermatitis. Infect. Immun. 2014, 82 (8), 3359-3373, doi: 10.1128/IAI.02077-14, PMID: 24866801.
18. *Kennedy R., Lappin D. F., Dixon P. M., Buijs M. J., Zaura E., Crielaard W., et al.*: The microbiome associated with equine periodontitis and oral health. Vet. Res. 2016, 47 (1), 49, doi: 10.1186/s13567-016-0333-1, PMID: 27080859.
19. *Nielsen M. W., Strube M. L., Isbrand A., Al-Medrasi W. D. H. M., Boye M., Jensen T. K., et al.*: Potential bacterial core species associated with digital dermatitis in cattle herds identified by molecular profiling of interdigital skin samples. Vet. Microbiol. 2016, 186, 139-149, doi: 10.1016/j.vetmic.2016.03.003, PMID: 27016768.
20. *Jang J., Hur H. G., Sadowsky M. J., Byappanahalli M. N., Yan T., Ishii S.*: Environmental Escherichia coli: ecology and public health implications – a review. J. Appl. Microbiol. 2017, 123, (3), 570-581, doi: 10.1111/jam.13468, PMID: 28383815.
21. *Rochelle-Newall E., Nguyen T. M. H., Le T. P. Q., Sengtaheuanghoung O., Ribolzi O.*: A short review of fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions. Front. Microbiol. 2015, 6, 308, doi: 10.3389/fmicb.2015.00308, PMID: 25926821.
22. *Refaai W., Van Aert M., Abd El-Aal A. M., Behery A. E., Opsomer G.*: Infectious diseases causing lameness in cattle with a main emphasis on digital dermatitis (Mortellaro disease). Livest Sci. 2013, 156 (1-3), 53-63, doi: 10.1016/j.livsci.2013.06.004.
23. *Barati A., Ghaderpour A., Chew L. L., Bong C. W., Thong K. L., Chong V. C., et al.*: Isolation and characterization of aquatic-borne Klebsiella pneumoniae from tropical estuaries in Malaysia. Int. J. Environ Res. Public Health. 2016, 13 (4), 426, doi: 10.3390/ijerph13040426, PMID: 27092516.
24. *Seidler R. J., Knittel M. D., Brown C.*: Potential pathogens in the environment: cultural reactions and nucleic acid studies on Klebsiella pneumoniae from clinical and environmental sources. Appl. Microbiol. 1975, 29 (6), 819-825, PMID: 1094000.
25. *Berrazeg M., Diene S. M., Drissi M., Kempf M., Richet H., Landraud L., et al.*: Biotyping of multidrug-resistant Klebsiella pneumoniae clinical isolates from France and Algeria using MALDI-TOF MS. PLoS One 2013, 8 (4), e61428, doi: 10.1371/journal.pone.0061428, PMID: 23620754.
26. *Dahmen S., Métayer V., Gay E., Madec J. Y., Haenni M.*: Characterization of extended-spectrum beta-lactamase (ESBL)-carrying plasmids and clones of Enterobacteriaceae causing cattle mastitis in France. Vet. Microbiol. 2013, 162 (2-4), 793-799, doi: 10.1016/j.vetmic.2012.10.015, PMID: 23127568.
27. *Munoz M. A., Welcome F. L., Schukken Y. H., Zadoks R. N.*: Molecular epidemiology of two Klebsiella pneumoniae mastitis outbreaks on a dairy farm in New York State. J. Clin. Microbiol. 2007, 45 (12), 3964-3971, doi: 10.1128/JCM.00795-07, PMID: 17928424.
28. *Ohnishi M., Okatani A. T., Harada K., Sawada T., Marumo K., Murakami M., et al.*: Genetic characteristics of CTX-M-type extended-spectrum-β-lactamase (ESBL)-producing Enterobacteriaceae involved in mastitis cases on Japanese dairy farms, 2007 to 2011. J. Clin. Microbiol. 2013, 51 (9), 3117-3122, doi: 10.1128/JCM.00920-13, PMID: 23843488.
29. *Wu X., Liu J., Feng J., Shabbir M. A. B., Feng Y., Guo R., et al.*: Epidemiology, environmental risks, virulence, and resistance determinants of Klebsiella pneumoniae from dairy cows in Hubei, China. Front. Microbiol. 2022, 13, 858799, doi: 10.3389/fmicb.2022.858799, PMID: 35603893.
30. *Karaiskos I., Giamarellou H.*: Multidrug-resistant and extensively drug-resistant Gram-negative pathogens: current and emerging therapeutic approaches. Expert Opin. Pharmacother. 2014, 15 (10), 1351-1370, doi: 10.1517/14656566.2014.914172, PMID: 24766095.
31. *El-Mahallawy H. A., Hassan S. S., El-Wakil M., Moneer M. M.*: Bacteremia due to ESKAPE pathogens: an emerging problem in cancer patients. J. Egypt Natl. Canc. Inst. 2016, 28 (3), 157-162, doi: 10.1016/j.jnci.2016.05.002, PMID: 27268592.
32. *Leyden J., McGinley K. J., Nordstrom K. M., Webster G. F.*: Skin microflora. Dermatol. Clin. 1987, 5 (1), 15-23, PMID: 3815993.
33. *Andonova M., Urumova V.*: Immune surveillance mechanisms of the skin against the stealth infection strategy of Pseudomonas aeruginosa – Review. Comp. Immunol. Microbiol. Infect. Dis. 2013, 36 (5), 433-448, doi: 10.1016/j.cimid.2013.06.002, PMID: 23838118.
34. *Al-Tememe T. M. K., Abbas B A.*: Molecular detection and phylogenetic analysis of Pseudomonas aeruginosa isolated from some infected and healthy ruminants in Basrah, Iraq. Arch. Razi. Inst. 2022, 77 (2), 537-544. doi: 10.22092/ARI.2022.357802.2099, PMID: 36284961.
35. *Chaudhary A., Ketkar O. A., Irfan S., Rana V., Rahi P., Deshmukh R., et al.*: Genomic insights into Omega-3 polyunsaturated fatty acid producing Shewanella sp. N2AIL from fish gut. Biology (Basel). 2022, 11 (5), 632, doi: 10.3390/biology11050632, PMID: 35625360.
36. *Vignier N., Barreau M., Olive C., Baubion E., Theodose R., Hochedez P., et al.*: Human infection with Shewanella putrefaciens and S. algae: report of 16 cases in Martinique and review of the literature. Am. J. Tro.p Med. Hyg. 2013, 89 (1), 151-156, doi: 10.4269/ajtmh.13-0055, PMID: 23690548.
37. *Kueh C. S. W., Kutarski P., Brunton M.*: Contaminated marine wounds – the risk of acquiring acute bacterial infection from marine recreational beaches. J. Appl. Bacteriol. 1992, 73 (5), 412-420, doi: 10.1111/j.1365-2672.1992.tb05001.x, PMID: 1468994.
38. *Bulut C., Ertem G. T., Gökcek C., Tulek N., Bayar M. A., Karakoc E.*: A rare cause of wound infection: Shewanella putrefaciens. Scand. J. Infect. Dis. 2004, 36 (9), 692-694, doi: 10.1080/00365540410022620, PMID: 15370659.
39. *Ceylan E., Berktas M., Ağaoğlu Z.*: The occurrence and antibiotic resistance of motile Aeromonas in livestock. Trop. Anim. Health Prod. 2009, 41 (2), 199-204, doi: 10.1007/s11250-008-9175-9, PMID: 18481193.
40. *Vivas J., Carracedo B., Riaño J., Razquin B. E., López-Fierro P., Acosta F., et al.*: Behavior of an Aeromonas hydrophila aroA live vaccine in water microcosms. Appl. Environ. Microbiol. 2004, 70 (5), 2702-2708, doi: 10.1128/AEM.70.5.2702-2708.2004, PMID: 15128521.
41. *Kayalvizhi N., Anthony T., Gunasekaran P.*: Characterization of the predominant bacteria associated with sheep and goat skin. J. Am. Leather Chem. Assoc. 2008, 103 (6), 194-201.
42. *Maughan H., Van der Auwera G.*: Bacillus taxonomy in the genomic era finds phenotypes to be essential though often misleading. Infect. Genet. Evol. 2011, 11 (5), 789-797, doi: 10.1016/j.meegid.2011.02.001, PMID: 21334463.
43. *Zinicola M., Higgins H., Lima S., Machado V., Guard C., Bicalho R.*: Shotgun metagenomic sequencing reveals functional genes and microbiome associated with bovine digital dermatitis. PLoS One 2015, 10 (7), e0133674, doi: 10.1371/journal.pone.0133674, PMID: 26193110.
44. *Rodrigues Hoffmann A.*: The cutaneous ecosystem: the roles of the skin microbiome in health and its association with inflammatory skin conditions in humans and animals. Vet. Dermatol. 2017, 28 (1), 60-e15, doi: 10.1111/vde.12408, PMID: 28133874.
45. *Jackson T. A., Pearson J. F., Young S. D., Armstrong J., O'Callaghan M.*: Abundance and distribution of microbial populations in sheep fleece. N. Z. J. Agric. Res. 2002, 45 (1), 49-55, doi: 10.1080/00288233.2002.9513492.
46. *Hurst C. J.*: Opportunistic bacteria associated with mammalian livestock disease, [in:] Hurst C. J. (ed.): The Encapsulation of Microbiota. Cham: Springer 2018, p. 185-238, doi: 10.1007/978-3-319-92373-4_6.
47. *Heimer S. R., Morrison D., Gilmore M. S.*: The enterococci, [in:] Rosenberg E., DeLong E. F., Lory S., Stackebrandt E., Thompson F. (eds.): Molecular Medical Microbiology, 2nd ed. Elsevier 2014, p. 717-730, doi: 10.1016/B978-0-12-397169-2.00039-1.
48. *Langworth B. F.*: Fusobacterium necrophorum: its characteristics and role as an animal pathogen. Bacteriol. Rev. 1977, 41 (2), 373-390, PMID: 883929.
49. *Zaura E., Keijser B. J., Huse S. M., Crielaard W.*: Defining the healthy "core microbiome" of oral microbial communities. BMC Microbiol. 2009, 9, 259, doi: 10.1186/1471-2180-9-259, PMID: 20003481.
50. *Tadepalli S., Narayanan S. K., Stewart G. C., Chengappa M. M., Nagaraja T. G.*: Fusobacterium necrophorum: a ruminal bacterium that invades liver to cause abscesses in cattle. Anaerobe 2009, 15 (1-2), 36-43, doi: 10.1016/j.anaerobe.2008.05.005, PMID: 18595747.
51. *Nagaraja T. G., Narayanan S. K., Stewart G. C., Chengappa M. M.*: Fusobacterium necrophorum infections in animals: pathogenesis and pathogenic mechanisms. Anaerobe 2005, 11 (4), 239-246, doi: 10.1016/j.anaerobe.2005.01.007, PMID: 16125078.
52. *Locher I., Greber D., Holdener K., Lüchinger R., Haerdi-Landerer C., Schüpbach-Regula G., et al.*: Longitudinal Dichelobacter nodosus status in 9 sheep flocks free from clinical footrot. Small Ruminant Res. 2015, 132, 128-132, doi: 10.1016/j.smallrumres.2015.10.021.

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