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CLINICAL AND BACTERIOLOGICAL STUDY REGARDING A DEEP CHRONIC GOAT'S DERMATITIS

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Abstract

Our study reveals the association between a chronic local-generalized clinical hard-skin infection in a 2-year-old Sannen goat and pathogens identified from lesions. The particular infection consists of a deep haemorrhagic-purulent, non-contagious, ineffectively treated dermatitis. The open skin lesions were infected with a mixed microbial flora. It was therefore difficult to identify the actual pathogenic microorganism necessary for effective and specific treatment. By conventional bacteriological examinations, we identified three bacterial pathogens: a haemolytic strain of Staphylococcus, a non-haemolytic strain of Corynebacterium and a haemolytic and highly proteolytic strain of Trueperella pyogenes. We also isolated many other germs, including Gram negatives that do not ferment lactose. For Staphylococcus and Corynebacterium, we assessed antibiotic susceptibility by the Kirby-Bauer diffusimetric method. Through local and general antibiotic treatment (beta-lactams, aminoglycosides), associated with a stimulation of the immune system and prevention of possible secondary liver disease, an improvement in housing conditions also improved clinical condition, appetite, livability and wound healing rate.

Key words*: dermatitis, goat, Staphylococcus, Corynebacterium, Trueperella.*

INTRODUCTION

Many bacterial infections can be distinguished in the pathology of small ruminants. Some of them affect all tissues - generalized infections, others are localized. For local skin infections, with deep and multiple, disseminated lesions, the pathogenic ability of the bacteria to synthesize and release enzymes/exotoxins affects the clinical condition: weakness, altered appetite, functional impairment of milk production, etc.

In goat pathology, staphylococcal infections, *Corynebacterium* infections, also Grips bacillus infections (*Trueperella pyogenes*), including mixed infections can be identified.

Staphylococcus infectious are frequent and dangerous, a real problem in veterinary medicine. Patogenic *Staphylococcus* strains cause clinically various forms, more or less aggressive, such: pustules, haemorrhagic lesions or discrete lesions. In goats, *Staphylococcus* infections can be secondary to contagious pustular dermatitis (e.g. parapox viral infection), or the integumentary infections involves the different predisposing factors. Acute clinical mastitis is associated with *Staphylococcus aureus* (Arteche-Villasol et al., 2022; Lima et al., 2020). Staphylococcal exotoxin production appeared to be a consequent event inducing the evolution to gangrenous mastitis (Rainard et al., 2018).

Staphylococcus aureus and *Corynebacterium* are responsible for liver abscesses, thus being a possible common complication in small ruminants (Rosa et al., 1989; Tadayon et al. 1980). One of the most recognizable signs of liver dysfunction in most species is icterus. However, ruminants often do not become jaundiced even when there is severe hepatocellular dysfunction (Fetcher, 1983).

The studies shown that majority of *S. aureus* with the highest rate of resistance to penicillin (Mechesso et al., 2021). Several studies support enrofloxacin therapy, being the right choice in this case (Polveiro et al., 2021).

Trueperella pyogenes is an opportunistic, but hardly pathogen, that causes suppurative deep infections in animals. Also, it can do the complications of a minor wound. Similar with *Trueperella*, *Corynebacterium* pathogenic

strains can cause persistent pyogenic infections, difficult to treat without a specific diagnostic.
In this paper, we propose a complex analysis, by clinical and paraclinical tests, of a goat with a particular deep and severe skin infection, located on the metapodial areas; thick crusts are also present on the mammary tegument and on the external pinna of the ears.

MATERIALS AND METHODS

Our clinical and microbiological analysis were realized in November-December 2023.
Clinical examination was permanently associated with paraclinical tests on this patient: complete blood count, cytology, and microbiological diagnostic.
All animals from the farm, including the goat, were up to date with preventive actions (vaccinations, internal-external deworming). For monitoring the herd, we took blood samples from several individuals. Only one animal has shown this pathology.

Clinical examination

Normally, the skin is constantly exposed to different microorganisms - real pathogenic, opportunistic, non-pathogenic (Figure 1). Different injuries of the skin barrier allow bacteria or other microbes to proliferate and penetrate deep into the lower layers of the skin (Faccin, 2023).
Our clinical examination included an epidemiological investigation involving risk factors, epidemiological data, feed and shelter analysis.
Also, the clinical study included determination of age, body weight, body temperature, assessment of dehydration, appearance of mucous membranes and clinical examination.

Figure 1. Metapodial skin lesions of exterminated goat

Paraclinical diagnostic analysis

1. *Haematology and biochemistry tests.* Blood samples were collected from jugular vein puncture, using 21 G needles, heparin and K3EDTA vacutainers.
Haematological and biochemical tests were performed using the haematology analyser Abacus Junior Vet 5 (SUA) and the biochemistry analyser Arkray Spotchem EZ SP4430 (Japan) (Figure 2).
The results were compared to the available relevant literature data.

Figure 2. Abacus Junior Vet 5 and the biochemistry analyser Arkray Spotchem EZ SP4430

2. *Microbiology analysis.* The biologic samples were represented by thick crusts from the skin lesions, introduced in a sterile test-tube. Because the crusts were dry, we added into recipient sterile physiologic solution, and shacked on vortex for decompose the primary hard crust's structure.
About the microbiological diagnostic, we applied two conventional steps. The first was represented by a direct bacterioscopic exam of the pathological sample, on smears stained by Gram method, also by blue methylene simple variant. In the second part, we made cultural analysis, using special and usual culture media: Columbia sheep blood agar plates, MacConkey lactosed agar plates, Brain Hearth Infusion broth, coagulated horse serum blood tubes, nutrient agar and nutrient simple broth.
We inoculated the liquid samples on Columbia sheep blood agar, incubated at 37°C, in normal atmosphere, for 24-48 hours, but we continued the exam of the prime cultures - maintained on room temperature, during other few days.
From Columbia sheep blood agar, we selected the isolated colonies, especially the smooth haemolytic ones, also the non-haemolytic rough type colonies, transferred on sterile fresh culture media, for the secondary pure cultures. The pure cultures were examined macro- and microscopic, also few biochemical-enzymatic tests for establish the particular pathogenic properties of isolated strains.

We detected the haemolytic activity, also proteolytic properties on coagulated serum blood.
On secondary pure cultures we established the bacteria cells morphology, correlated with the macroscopic aspects, for obtain the typical germs properties.
Using the secondary cultures, decimal diluted, we performed the bacterium sensitivity test, by the antibiotesting, Kirby-Bauer method.

Local and general infection treatment
Although systemic and topical treatments have their usefulness, it is essential to manage risk factors to stop recurrences.
Supportive therapy was also provided together with a conservative two steps treatment. Therapy was initiated according to the antibiogram results.
We applied a two-step conventional therapy. The first was an injectable treatment with penicillin G procaine (Penstrep) in doses of 8 mg/kg penicillin and 10 mg/kg dihydrostreptomycin for 3 days, after which we decided to initiate treatment with enrofloxacin (Enroxil 5%) a daily dose of 50 mg/kg for a minimum of 5 days. To prevent secondary liver damage, dehydration and apathy, we resorted to fluid therapy and liver support.
For the secondary part of the treatment (local treatment) we chose neomycin spray and chlorhexidine washes twice a day. Complete healing was achieved after 62 days.
The first choice was penicillin for 3 days, then enrofloxacin to which both *Staphylococcus* and *Corynebacterium* were sensitive. Enrofloxacin is a fluoroquinolone, which acts by inhibiting DNA gyrase which causes bacterial cell division to be blocked. They are also known to have direct effects on the immune system.
As abscesses are known to occur in internal organs, with specificity in small ruminants for the liver area, secondary to *Corynebacterium*.

RESULTS AND DISCUSSIONS

Clinical examination
We identified the next general aspects of our case: approximate age of 2 years, body weight 23 kg, temperature 38.2°C, degree of dehydration > 12%, sticky whitish mucous membranes. Clinical examination on machines indicated increased intensity of cardiac noises, vesicular murmur at pulmonary auscultation, reaction to abdominal palpation on the hepatic projection area, ruminal noises present, defecation and normal urination. Decreased appetite and temperature were the most important indications for chronic secondary systemic involvement.
Multiple nodular lesions were identified on the skin, with active bleeding, pungent smell and myasis. The lesions tend to desquamation, with the extension of the proliferation area to the udder, posterior portion and ears. Open lesions showed numerous thick bloody scabs, especially on the limbs. In our case we included a mastitis with bacterial origin, in asymptomatic stage, most likely consecutive to chronic evolution.
The particular infection consists of a deep haemorrhagic-purulent, non-contagious, ineffectively treated dermatitis.
In conclusion, clinical examination revealed a deteriorated general condition, pronounced weakness, inadequate maintenance, chronic bad-smelling haemorrhagic lesions on the hind limbs. The goat showed multifocal alopecia with ulcers, erosions and scabs, predominantly on the dorsal portion. The skin lesions were pruritic, firm, demarcated, haemorrhagic and pigmented.
Cytology and biochemical results
Complete blood count showed ***chronic neutrophilia***, ***lymphocytic reaction*** and ***increased blood platelets***.
On the basis of these diagnostic tools, the diagnosis of chronic inflammatory skin disease complicated with recurrent bacteria was established. In these cases, the most important diagnostic test is the microbiological one.
Microbiology analysis results
The general appearance of the hydrated sample showed the presence of grey-greenish portions mixed with red parts (Figure 3). The odour was repulsive, ihorous.
Macroscopic aspects of primary cultures
The primary culture on Columbia sheep blood agar was very abundant, mixed: (1) a large number of medium size colonies, pigmented - grey-withe-yellow, smooth surface, circular edges, convex to flat profile, surrounded by a complete haemolysis; (2) many tiny colonies, rough surface, flat, non-haemolytic, grey

colour, with a medium to high consistence. After few days, we detected the presence of (3) the very small colonies, smooth type, surrounded by a narrow weak beta haemolysis area. We appreciated that the all three types mentioned colonies represent the codominant microflora. For other colonies types, we considered they correspond with saprophytic microbes.

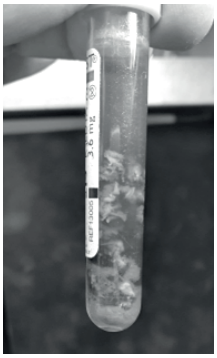

Figure 3. Biological sample, hydrated and mixed

On MacConkey lactosed agar, the cultures showed an abundant flora, with medium (a) and little (b) size colonies, smooth type; all of them are lactose-negatives.

Macroscopic aspects of secondary cultures

On pure secondary cultures, we observed the characteristic aspects of yellowish pigmented Staphylococcus on nutrient agar, and a thin ring and low turbidity in nutrient broth, that correspond with type (1) colonies; rough type, non-pigmented, opaque colonies, and a pellicule, medium to low turbidity - for type (2) colonies, on simple culture media; a low turbidity, a medium to large pulverulent sediment, non-adherent, on Brain Hearth Infusion broth, after few days of incubation, for type (3) colonies (Grips bacillus).

For lactose negative strains, from MacConkey agar plate, we obtained on nutrient slant agar smooth type, non-pigmented colonies, medium (a), and little (b) size, respectively, with a medium to high turbidity on nutrient broth. For (a) type, we observed a pellicule on the broth surface.

Microscopic analysis

The direct analysis of the samples, by Gram staining method, revealed: (1) many *Corynebacterium*-like Gram-positive polymorphic cocobacilli, arranged typically diplo V shape, and Chinese characters; (2) Gram-positive cocci, bunch of grapes arranged; (3) Gram-negative medium size isolated cocobacilli were also present, but in low number; (4) cellular debris, and the pycnotic nucleus of leucocytes.

In blue methylene staining method, we remarked similarities with the Gram stained smears, but a particular aspect of the *Corynebacterium*-like cells was significant: a specific irregular aspect of stained cytoplasm, by the presence of disseminated inclusion, and the biggest ones were at the extremity of bacterium, constantly.

We examined smears, staining by two methods, from the secondary pure cultures, too.

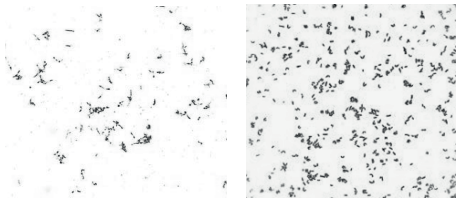

Figure 4. *Trueperella pyogenes* bacteria cells (left), *Corynebacterium* (right), Gram staining method, 1000x

The morphology of stained *Staphylococcus* revealed the Gram positive cocci, specifically arranged. For *Corynebacterium*, and *Trueperella*, Gram-positive bacteria, we detected many similarities, but *Trueperella* cells were more polymorphic, thin cells, with many cytoplasmic inclusions, and the dominant arrangement was Chinese characters (Figure 4).

Biochemical-enzymatic properties

The proteolytic character of small haemolytic beta colonies was very intense on coagulated serum blood cells. Indeed, within a short time - one day at 37°C, another few days at laboratory temperature, the serum was transformed almost entirely into a liquid consistency (Figure 5). One can easily correlate this breakdown of total serum proteins with the high pathogenicity of *Trueperella pyogenes*, as it can similarly break down proteinaceous tissues, such as the deep integument, *in vivo* layers. We add other enzymatic previous test - haemolysis.

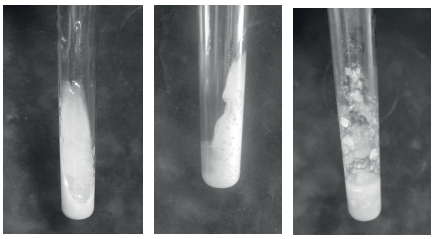

Figure 5. Intense proteolytic action of *Trueperella pyogenes* on coagulated blood from horse serum

By antibiotic susceptibility testing, we determined the susceptibility of *Staphylococcus* and, separately, *Corynebacterium* to some antibiotics (Figure 6).

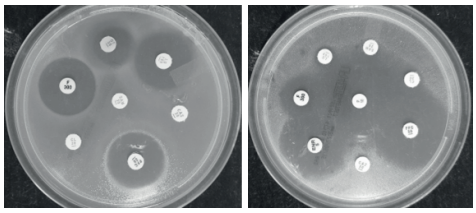

Figure 6. Antibiotic testing by Kirby-Bauer method

The *Staphylococcus* strain exhibited (Table 1) full resistance to Methicillin, which includes similar properties to the antibiotic beta-lactams. We obtained good susceptibility to fluoroquinolones as well as piperacillin, rifampicin, florfenicol and nitrofurantoin. A medium sensitivity to oxytetracycline was a good result, as it can be used directly on infected areas. The same important information corresponds with nitrofurantoin, as it can be used directly on infected tissues.

Table 1. Antibiotic test results for *Staphylococcus*

	ANTIBIOTIC SUBSTANCES	THE RESULTS
1.	METHICILLIN	R
2.	AMPICILLINE-SULBACTAM	R
3.	CEFALEXINE	R
4.	CEFTAZIDIME	R
5.	CEFUROXIM	R
6.	**PIPERACILIN**	**S**
7.	**RIFAMPICINE**	**S**
8.	OXYTETRACYCLINE	MS
9.	LINCOSPECTINE	MS
10.	**ENROFLOXACINE**	**S**
11.	**PEFLOXACINE**	**S**
12.	**OFLOXACINE**	**S**
13.	**FLORFENICOL**	**S**
14.	**NITROFURANTOINE**	**S**

Antibiotic testing of *Corynebacterium* strain (Table 2) showed many opportunities, such as susceptibility to penicillin, similar enrofloxacin, neomycin, bacitracin and nitrofurantoin.

Table 2. Antibiotic test results for *Corynebacterium*

	ANTIBIOTIC SUBSTANCES	THE RESULTS
1.	**PENICILLIN**	**S**
2.	AMPICILLINE-SULBACTAM	R
3.	CEFALEXINE	R
4.	CEFTAZIDIME	R
5.	CEFUROXIM	R
6.	**PIPERACILIN**	**S**
7.	**FLORFENICOL**	**S**
8.	OXYTETRACYCLINE	R
9.	LINCOSPECTINE	R
10.	ERITROMICINE	R
11.	**ENROFLOXACINE**	**S**
12.	**NEOMICINE**	**S**
13.	POLIMIXINE B	R
14.	**BACITRACINE**	**S**
15.	**NITROFURANTOINE**	**S**

We highlighted the possibility of a local antimicrobial therapy in combination with systemic antibiotics. Another aspect to be mentioned is the respect of common antibiotic susceptibility for both bacterial strains.

Treatment results

After microbiological diagnosis, response to therapy was initially rapid, followed by a period of slow healing. Peak clinical response was generally observed within 2 weeks of starting treatment.

Because microbiological diagnosis is complicated and involves different bacteria, we developed a continuity treatment plan by combining antibiotics to which they are sensitive. This animal was isolated from the others, then removed from the breeding plan and milked.

The clinical response to enrofloxacin was rapid but long-term and chronic pathology was associated with dramatic clinical signs. Although the animal was diagnosed at a very advanced stage, the efficacy of treatment was confirmed from the first day, with an improvement in general condition identified.

Healing was slow, possibly due to chronic pathology and multiple bacterial skin diseases.

Healing was complete 62 days after the start of treatment.

CONCLUSIONS

Through complex diagnostic tools we can provide some relevant conclusions:

1. The clinical exam is essential for establish the character of the disease.
2. The cytological exam and the blood tests provide many important data regarding the immunologic reactions of the patient.
3. Microbiology can identify the pathogenic microorganism involved in the dermatitis lesions. Also, a specific therapy can be correctly conducted according to the antibiotic sensitivity testing results.
4. Both topic and general complex treatment are required in this type of dermatitis for a good management of the case.

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