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A new pathology of subdermal abscesses in goats in southwestern Mexico

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Abstract: *Staphylococcus hominis is for the first time reported here as an agent causing superficial abscesses in goats. Five replacement doelingsin a stabled dairy goat herd in La Paz, Mexico were detected with skin nodules located in the neck and the submandibular area. These nodules developed after 7 days in abscesses resembling caseous lymphadenitis lesions. Body temperature was found increased in infected kids in comparison with not infected control kids(40.5°C±0.3 vs. 39.6°C ±0.2). Blood cell counts of leucocytes (17.3±3.7 vs 10.2±2.7.), neutrophils (56.7±10.8 vs 39.9±8.7), and lymphocytes (36.8±11.3 vs 55.7±8.09) were found increased in abscessed kidsas well (P<0.05). Cultures of abscess contents, isolation, and biochemical identification of the pathogen revealed that the coagulase-negative S.hominiswas the causing agent. The 16S rDNAsequence confirmed the presence of S.hominis. The necropsy and the pathological analyses indicated the occurrence of granulomas with infiltrates of macrophages, lymphocytes, plasm cells and fibroblasts organized to encapsulate the granuloma.*

Keywords*:pyogenic bacteria, staphylococci, goats, abscesses*

I. Introduction

Abscesses are regarded as a major problem for small ruminant health and welfare [1]. In these species, abscesses may cause anemia and emaciation that result in low productive and reproductive performance, as well as economic losses due to slaughter and total or partial carcass condemnation. Currently, caseous lymphadenitis caused by *Corynebacterium pseudotuberculosis*is considered as the most important disease producing abscesses in small ruminant operations all over the world [2]. However, other microorganisms such as Streptococcus, Staphylococcus, Pseudomonas (among others) have been also isolated from sheep and goat abscesses as well [1].Recently, Coagulase-Negative Staphylococci (CNS) have been involved in diverse pathologies in small and large ruminants [3]. Although CNS are not considered as primary pathogens in livestock, there is growing evidence that they are involved in various infectious processes [4] including mastitis and skin infections in ruminants [5].Several species of Staphylococcus, including *S. hyicus* subsp. *hyicus, S. simulans, S. haemolyticus, S. epidermidis, S. Lugdunensis, S. capitis, S. warneri, S. xylosus, S. cohnii*, and *S. saprophyticus*have been reported as abscess-producing agents[6,7], but evidence of the participation of *S. hominis* as a primary pathogen causing abscesses in goats is lacking so far. The objectives of this work were to identify a strain of *S. hominis* as an agent causing goat abscesses and to describe the clinical, hematological and pathological characteristics of an outbreak of superficial staphylococcal abscesses in Mexico.

II. Material and methods

2.1 Animals and housing

The infected goats were replacement females(five-month old, average weight 25±3 kg, Saanen × Nubian crossbred kids, *n*=5)in a stabled goat herd in La Paz, Baja California Sur, Mexico, under climate hot and dry. The breeding stock consisting of 91 goats of different ages, which were reared in the vicinity of equally stabled sheep (*n*= 80). Goats and sheep were maintained permanently in open, shaded pens equipped with feeders and water drinkers. Growing and adult animals were injected with A,D,E vitamins and ivermectin at three-month intervals. The open housing allowed eventual presence of wild birds and rodents. All the animals received a diet composed of alfalfa hay (3.0% of body weight) supplemented with concentrate. Routines of pen cleansing, withdrawal of wasting materials and change of beds were practiced biweekly.

2.2 Disease detection and clinical exploration

Unusual cases of abscesses were recorded during the spring (April-June, 2014). The abscesses outbreak affected total of six out of 36 (17%) young goats born during the kidding season. In this herd, caseous

lymphadenitis was known to be prevalent, but the clinical signs did not correspond with the pathognomonic sites, abscess growth pattern or age at which lymphadenitis is typically distinguishable in small ruminants[8] .Development of the abscesses was followed-up during a 30 days period after the initial detection. Eight female kids (similar weight and age without clinical signs before and after the study period) belonging to the herd were taken as not infected reference controls.

2.3 Tissue sampling

Blood samples were taken from infected and not infected kids using heparinized Vacutainer tubes (BD, Mexico) by puncture of the jugular vein at two-weekintervals (day of initial detection of the abscess= Day 0) until necropsy was carried out (Day 30).The abscessed area was depilated and disinfected with an iodine tincture. Samples of abscess contents were obtained from the kids with the aid of sterile syringes. Samples were conserved at 4°C during transportation and immediately processed for further microbiological and molecular analyses.

2.4 Blood parameters

Blood samples were immediately processed using a HemaTrue Hematology Analyzer (Heska Lab. Systems, Loveland Co.). Blood parameters analyzed were: erythrocytes count, hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), leucocyte total count, neutrophils %, eosinophils %, basophils %, lymphocytes % and monocytes %.

2.5 Microbiological cultures

Isolates were cultured in blood, chocolate, mannitol agars and trypticase soy broth at 37°C for 24 h. Direct smears of bacterial growth spots and Gram staining revealed the presence of abundant cocci colonies. Biochemical tests catalase, coagulase, and novobiocinwere carried out on the cultures. The bacterial inhibition assay in novobiocinwas also carried out according to the procedures described by Hariharan*et al*[9].

2.6 Molecular identification of the etiological agent

Total DNA extraction and molecular identification from several isolates was carried out as described previously [10]. The 16S rRNA gene sequence of each isolate was amplified using pA (5'-AGAGTTTGATCCTGGCTCAG-3') as forward and PH* (5'-AAGGAGGTGATCCAGCCGCA-3') as reverse primers. The PCR reaction mixture consisting of 1x PCR enzyme buffer, 1.5 mM of MgCl2, 0.2 mM of dNTP's mix, 0.5 µM of each primer, 0.03 U/µL of Taq polymerase and 1 ng/µL of template DNA. The thermocycler program was as follows: 95ºC for 4min; 30 cycles of 94ºC for 45 s, 56ºC for 45 s, and 72ºC for 1 min; and a final extension step at 72ºC for 5 min. The PCR products were analyzed by electrophoresis on a 1.0 % agarose gel stained with syber green in 1X TAE buffer at 100 V for 45min. The bands were visualized under UV trans-illuminator. The sizes of DNA fragments were estimated (~1.6 kb) using a standard 1kb DNA ladder (Norgenbiotek Corporation, Canada) and sequenced (Macrogen, Korea). The 16S rDNA sequences of isolated strains were compared to sequences from type strains held in GenBank DNA database using BLAST.

2.7 Histopathological analysis

The infected kidswere slaughtered in a local municipal abattoir one month after the initial detection of the abscesses. Tissue samples including: skin adjacent to the lessions, abscess, lymph nodes and surrounding muscle were collected at the necropsy. The samples were fixed in Davison solution, dehydrated and embedded in paraffin. Four-micron thick cuts were prepared with the aid of a microtome. Cuts were then stained with eosin-hematoxylin and mounted on glass slides for posterior histopathological analysis and description.The histopathological lesions were analyzed according to origin of tissue, lesion, epidermal hyperplasia, existence of necrosis, and type of inflammation [11].

Statistical analysis

Mean body temperature and values of blood parameters were compared between groups of not infected vs. infected kids by a student's t test for independent samples and groups having unequal sample size [12].

III. Results

3.1 Clinical exploration

The abscesses were located in the ventral area of the neck (round-shaped) and the submandibular area (diffuse-shaped). The *in situ* palpation revealed a firm consistency of nodular structures in the subdermal tissue. Average rectal body temperature was 40.5 º C ± 0.3 in infected and 39.5 º C ± 0.2 in not infectedkids (p<0.05).

3.2Blood parameters

Values of blood parameters erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, eosinophils, basophils, and monocytes were observedin the normal ranges for goats (Table 1). By contrast, total leukocyte count was found increased (leukocytosis) in both, not infected and infected kids at the beginning of the sampling period. At the end of the sampling period, only the infected kids still showed leukocytosis (17.3±3.7) respective to the not infected kids (10.2±2.7), which returned to the normal range. Neutrophil count was seen augmented in infected kids at all samplings. Conversely, infected kids had lower values of lymphocyte counts (lymphopenia) than not infected kids or the reference values.

3.3Pathogen identification

Staphylococci grown in the culture media were demonstrated to be coagulase-negative. The bacterial inhibition assay in novobiocin measured 14-15 mm, which indicated the identity of *S. hominis*[9].The PCR analysis confirmed the presence (Fig. 1-f) of *Staphylococcus hominis* (99%).

3.4Histopathological description of the lesions

Fibrous connective tissue was always found in the skin samples. In abscess tissue, the presence of granulomas with abundant calcified spots was evident. These calcified spots were surrounded by abundant epithelioidal cells, scarce giant cells, macrophages and reactive fibroblasts organized to encapsulate the calcified spots. In the cortex of lymph node tissue, proliferation of cells from the reticuloendothelial system (histiocytes, macrophages), and dendritic cells were observed. The germ nucleus was replaced by this cell proliferation, leading to moderate lymphoid atrophy. In muscular tissue, inflammatory infiltrates surrounding the muscle fibers were observed. Inflammatory infiltrate consisted mainly of macrophages and lymphocytes.

IV. Discussion

The disease under study was characterized by a relatively quick formation of abscesses in the subdermal tissue of the neck and submandibular areas, although lymphatic nodes and intramuscular tissues were affected in a lesser extent as well. These lesions observed were similar to those described for Morel disease [13], caused by *Staphylococcus aureus* subsp. *Anaerobius*which is endemic in Africa and Asia countries. Moreover, Morel disease affects mainly young goats and full clinical signs are developed after three weeks. This is a relatively short period compared to caseous lymphadenitis which affects older animals, showing clinical signs after an incubation period of 2 to 6 months[14].Increases in both, leukocytosis and neutrofilia were observed in not infected kids at the beginning of the sampling period. These higher values may be due to a physiological leukocytosis associated to handling and separation of the kids from the herd for tissue sampling [15].

In general, CNS are considered as non-pathogenic in livestock, but rather as contaminant or colonizer microorganisms. However, when trauma, inoculation or implantation of foreign objects occurs, these bacteria may cross the skin barrier. In such cases, become pathogenic due to their capability of adhesion to live tissues or to the surface of the implanted devices. According to Bochniarz*et al.*[16], the pathogenic mechanisms of CNS consist in both, invasiveness and toxicity. In particular, CNS are able to permeate through protective barriers, to adhere to host's cells and produce biofilms. Thus, when CNS become pathogenic, these bacteria evade the immune system, reproduce intensively and produce enzymes (proteases and hemolysins) and substances toxic for the host organism [16,17]. The leukocytosis observed in infected kids was very likely associated with a bacterial invasion. Abscess development, accompanied by inflammatory infiltrates and foci of necrosis coincided with lesions caused by *S. aureus*in mice and rabbits [18].

CNS are considered as the causing agent of an established infection, if the strain isolated is predominant or unique in the purulent drainage[19]. In the present work, since*S. hominis*was the only pathogen isolated from the abscess samples, we assumed it was the agent causing the outbreak of goat abscesses. This type of pathologies are rarely documented in the medical literature [19].

In conclusion, a definitive diagnosis of the etiological agent causing the abscesses was carried out by biochemical tests and then confirmed by the PCR analysis. Both, the clinical and histopathological studies contributed to describe this new pathology caused by the coagulase-negative *S. hominis* in goats.

Conflict of interests

The authors declare not to have any conflict of interests

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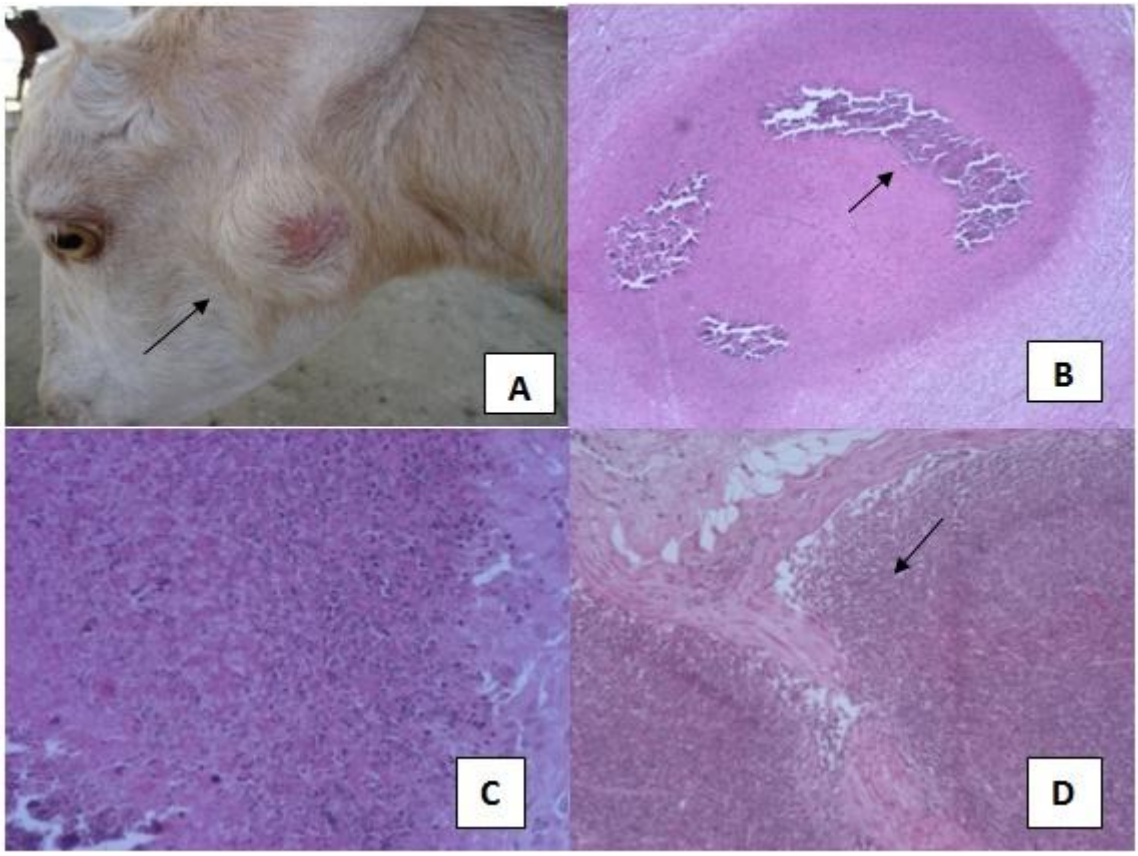
Table 1. Mean (±SD) blood parameters in goats with and without abscesses caused by *S. hominis* at the beginning and at the end of the sampling period.

Parameter	Normal range[1]	Initial[3]		Final	
		Not infected	Infected	Not infected	Infected
Erythrocytes, 10^6/µl	8-18	14.4±1.5[a]	16.8±1.0[b]	14.3±0.7[a]	15.4±1.5[b]
Hemoglobin, g/dL	8-12	10.6±0.6[a]	10.1±1.0[a]	8.9±0.6[a]	9.7±1.09[b]
Hematocrit %	22-28	36.2±1.8[a]	38.5±3.7[a]	34.02±2.3[a]	36.5±3.4[b]
MCV, fl	16-25	24.7±3.9[a]	22.9±2.6[a]	23.5±1.4[a]	23.7±2.2[a]
MCH, pg	5.2-8	7.8±0.9[a]	6.05±0.6[b]	6.18±0.3[b]	6.3±0.7[b]
MCHC, g/dL	30-36	27.8±1.6[a]	26.3±1.0[b]	26.7±1.1[b]	26.6±1.6[b]
Leukocytes, 10^3/µL	4-13	18.1 ±2.7[a]	16.5±4.4[a]	10.2±2.7[a]	17.3±3.7[b]
Neutrophils, %	30-48	33.7±3.4[a]	58.5±7.1[b]	39.9±8.6[a]	56.7±10.8[b]
Eosinophils, %	1-8	2.25±1.0[a]	3.98±3.0[a]	4.85±2.8[b]	3.53±3.3[a]
Basophils, %	0-1	0.87±0.6[a]	0.64±0.6[a]	0.77±0.5[a]	0.53±0.6[a]
Lymphocytes, %	50-70	60.2±3.0[a]	35.3±7.3[b]	55.7±8.1[a]	36.8±11.3[b]
Monocytes, %	0-4	0.75±0.5[a]	1.53±1.2[a]	0.62±0.5[ab]	2.4±2.14[b]

[1]Normal ranges of hematological reference in goats [20].
[2]Comparisons blood values between not infected (n=8) and infected kids (n=5) at the beginning vs. end of the sampling period. Means of blood parameters with different superscripts indicate significant statisticaldifferences (p<0.05).

Figure legends

Figure 1.A.-Location and appearance of submandibular subdermal abscesses caused by *S. hominis* in a goat kid. B.-Histological microcut of a granuloma in the abscess (Eosin-Hematoxylin staining) with central focal necrosis and calcification (100x). C.-Granuloma surrounded by epithelioidalcells, accompanied by scarce multinucleated giant cells and macrophages; fibrous connective tissue around the granuloma is evident (400x). D.-Histological microcut of a lymph node with moderate lymphoid atrophy and granuloma (100x).E.-Histological microcut of muscle adjacent to the abscess, showing zonal granulomatous myositis. F- Molecular identification of *S. hominis*with agarose gel showing the PCR products. Extraction of genomic DNA (1-3) and amplicons of gen 16S rARN(1a-3a).



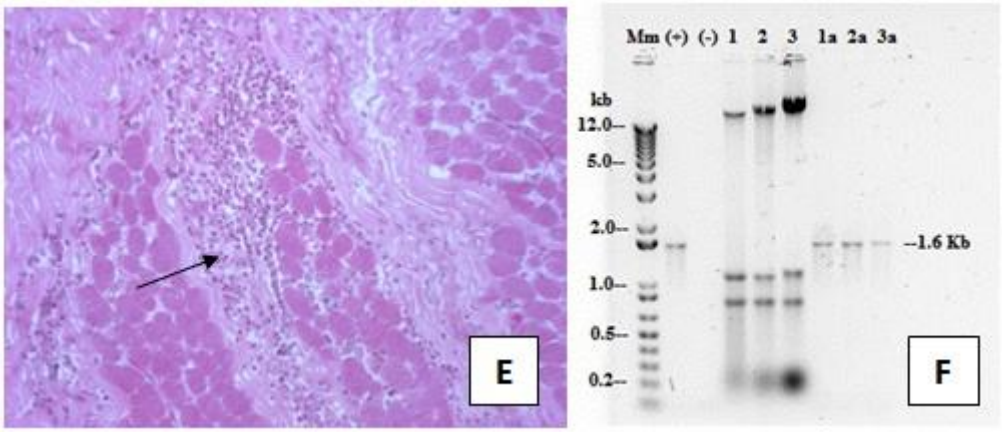


Figure 1.

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