CORYNEBACTERIUM PSEUDOTUBERCULOSIS EXPERIMENTAL INFECTION OF GOATS MAMMARY GLAND

J.W. Pinheiro Junior¹, A.A.F. Oliveira¹, F.S.F. Alves², L.B.G. Silva¹, S.S.A. Rabelo¹, R.A. Mota¹

¹Universidade Federal Rural de Pernambuco, Departamento de Medicina Veterinária, Rua Dom Manuel, s/n, CEP 52171-900, Recife, PE, Recife, Brazil. E-mail: jrwilton@bol.com.br

ABSTRACT

The objective of this study is to describe some aspects of the pathogeny of Corynebacterium pseudotuberculosis, isolated from a natural case of caseous lymphadenitis. The study was comprised with 2 groups of 3 animals each; inoculated with a C. pseudotuberculosis strain, group 1 (GI) via intradermal and group 2 (GII) via the mammary gland. The animals were clinically examined on a daily basis and hemograms carried out over a period of time at different intervals. Only 2 animals from GII showed changes in the clinical examination characterized by hyperthermia. All the animals in both groups developed papule and edema at the inoculation site. There were no significant alterations in the erythrogram but in the leucogram there was leucocytosis indicated by the presence of neutrophilia in 2 animals from GII. Results of the cellular analysis of the gland showed that 2 animals from GII developed acute mastitis. This experiment has proven that C. pseudotuberculosis, isolated from a natural infection of caseous lymphadenitis, could cause clinical mastitis, losses in goat keeping and that the expression cytology test could be used as an alternative diagnosis for mastitis.

KEY WORDS: Mastitis, caseous lymphadenitis, expression cytology.

INTRODUCTION

Goat rearing is a developing activity throughout the country, although there are a greater number of goat farmers in the semi-arid regions of the North East Brazil (Ribeiro et al., 2001). Some diseases, such as mastitis and caseous lymphadenitis, reduce production and productivity thus resulting in losses to the producers and risks to Public Health. Caseous lymphadenitis is a chronic disease with a suppurative characteristic and necrotic inflammation characterized by internal and external abscesses and is caused by Corynebacterium pseudotuberculosis (NF; ND, 1994). It causes economic...
losses by lowering milk and meat production, and the value of the hide, condemnation of carcasses and viscera are condemned, slaughtering of severely affected animals, veterinary expenses and labor costs in treating the abscesses and, in severe cases, death of the animal (Alves; Pinheiro, 1999).

Almeida et al. (2005), in their study in Pernambuco State, isolated C. pseudotuberculosis in 60% (3/5) of lactating goats. These had mastitis and concomitant caseous lesions in the supra mammary lymph node which is characteristic of the disease.

These diseases, isolated or in conjunction with other sanitary problems of small ruminants, contribute considerably to lowering the quality of the milk, meat and hide, thus leading to financial losses what could even turn the breeding venture unviable.

Considering the importance of goat breeding in Pernambuco State and the lack of regional research that contributes in clarifying the epidemiology and the disease control, the objective of this study is to evaluate some important aspects of the C. pseudotuberculosis pathogenicity in the mammary gland of milking goats.

MATERIAL AND METHODS

A strain of C. pseudotuberculosis obtained from a clinical case of caseous lymphadenitis of animals from Centro Nacional de Pesquisa em Caprinos was used in the experimental infection. The strain was identified according to the method recommended by Carter (1988). The infective dose was 3.6 x 10^7 units of formed colonies (Burrell, 1978).

Six cross breed goats in different stages of lactation, in good health condition, without any clinical sign of mastitis and with milk microbiological examination negative for C. pseudotuberculosis and other agents, were used in the experiment. The animals were kept in pens at the CNPC and were fed on elephant grass, corn flour ration and cotton pie, and had water ad libitum. The goats were separated into 2 groups of 3 animals each.

Using syringes and needles disposable, GI was infected via intradermal and GII was inoculated with 0.2 mL of infected material directly into the parenchyma of the udder and approximately 5 cm to the right of the supra mammary lymph node. The infective dose was 3.6 x 10^7 units of formed colonies (Burrell, 1978).

The clinical examination was carried out according to Rosemberg (1993) taking into account physiological parameters (body temperature, heart beat and respiratory rate), palpations of the superficial lymph nodes and mammary gland once a day, for one week of adaptation and the three weeks post infection (PI). Samples of milk were collected before inoculation to verify the state of health of the udder and after inoculation to recover the microorganism inoculated. These samples were seeded on agar base enriched with 8% sheep blood and incubated for 48 hours at 37º C. After this incubation period the morphological characteristics of the colonies were recorded and colonies smears stained by Gram technique according to Quin et al (1994).

Hemograms carried out according to Jain (1993) method were done for blood samples collected 72 and 8 hours before the inoculation and 48, 168, 326 and 672 hours post inoculation, comprising a total of 36 samples.

The procedure used in the expression cytology (EC) was adapted from Azira method (1976) in which, after the teat cleaning with alcohol 70%, slight pressure is applied with the thumb and index finger to strip the entire canal of the teat. The material obtained was placed on an opaque histology slide, fixed with absolute methyl alcohol for ten minutes then stained with Giemsa. These slides were examined for evaluating characteristics of stripping, composition and cell size. Cell counts were carried out on ten fields under the microscope and the mean cell count taken for the type of inflammation classification as well as cell component. The classification of the type of inflammation was carried out according to Cowell; Tyler (2002).

The animals were slaughtered on the 28th day post infection and samples of the supra mammary lymph nodes were collected for bacteriological and histopathological examination.

Statistical analysis was carried out on data of animals within the same group using the Minitab computer program. The data were tested for normality of residues and the degree of freedom in opting for the development the analysis by Nparl way analysis of non parametric variance and utilizing the Kruskal-Wallis test (Sampaio, 1998).

For results description the respective measurements of the central trend were used: mean and standard deviation for the erythrogram and mean, lower and superior limits for the leucogram. The level of significance used for rejecting HO (null hypothesis) was 5%.

The present study was approved by EMBRAPA Ethical Committee on March 2005.

RESULTS

Only 2 animals of GII had fever during the first 96 hours post infection. From the 5th day on, the animals had normal rectal temperature. However, one animal developed vital changes with tachycardia and tachypnea and showed physical alteration of lacteous secretion 48 hours PI. Some lumps were observed and

Corynebacterium pseudotuberculosis experimental infection of goats mammary gland.

A reduction in milk production and turning into a purulent viscose content after 72 hours. Apathy, loss of appetite and a reduction in food consumption 72 hours post infection, remaining 48 without eating, was also observed. The other 4 animals had no fever or any other changes in their general health status.

No significant differences were observed in the variables analyzed: haematocrit (Ht), erythrocytes (He) and hemoglobin (Hb) for GI and GII animals on the different sampling times (Table 1). However, there was a Hemoglobin decrease in the GI 48 hours PI. In the leucogram there was a significant difference in the total number of leucocytes was observed in both groups, 48 hours PI, characterizing a leucocytosis, with a decrease in number 168 hours PI. Analysis of the number of lymphocytes indicated an increase in GI forty-eight (48) hours PI with a decrease 168 hours PI.

Examination of the site of inoculation revealed papule formation 48 hours PI in all GI animals and transformation of this into pustule on day 5 PI and developing into an ulcer fairly soon after. All the GII animals developed edema at the site of inoculation. On day 20, GI animals showed ulcer cicatrisation preceded by a dry crust formation.

An increase in the supra mammary lymph nodes (lymphadenitis) was observed 48 hours post infection in all the inoculated animals except for goats number 013 and 066 (GI), which showed it 168 hours post infection.

Analysis of the gland cells of the infected animals revealed that for 2 GII animals more than 70% of the inflammatory cells were neutrophils, considered acute mastitis according to COWELL; TYLER (2002) criteria (Fig. 1). On the other hand, the neutrophils counts increased progressively over the post infection period for animals 68 and 119 and there was also an increase, predominantly in neutrophils in animal 180. The results for the EC are given in Table 3. It was not possible to carry out EC on animals 013 and 066 (GI) as these went dry during the period of adaptation.

Table 1 - Average values of the erythrogram of goats inoculated intradermically (GI) and intra-mammary (GII) with Corynebacterium pseudotuberculosis, CNPC, 2005.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group1 (hours)</th>
<th>Group2 (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>72</td>
<td>8</td>
</tr>
<tr>
<td>Hematocrito (%)</td>
<td>23.00</td>
<td>24.00</td>
</tr>
<tr>
<td>HCM (pg)</td>
<td>9.53</td>
<td>8.97</td>
</tr>
<tr>
<td>CHCM (g/dl)</td>
<td>33.77</td>
<td>31.67</td>
</tr>
<tr>
<td>He (x 10^6)</td>
<td>8.13</td>
<td>8.52</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>7.77</td>
<td>7.60</td>
</tr>
</tbody>
</table>

Table 2 - Leucogram median values of goats experimentally inoculated, intradermically (GI) and intramammary (GII) with Corynebacterium pseudotuberculosis, CNPC, 2005.

<table>
<thead>
<tr>
<th>Hours</th>
<th>Leucocytes</th>
<th>Variables</th>
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<tr>
<td></td>
<td></td>
<td>Segmented</td>
</tr>
<tr>
<td>GI</td>
<td>72</td>
<td>9050,00</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>10750,00</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>24400,00</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>24200,00</td>
</tr>
<tr>
<td></td>
<td>336</td>
<td>11850,00</td>
</tr>
<tr>
<td></td>
<td>672</td>
<td>9100,00</td>
</tr>
<tr>
<td>GII</td>
<td>72</td>
<td>7550,00</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6650,00</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>17200,00</td>
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<tr>
<td></td>
<td>168</td>
<td>13350,00</td>
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<td></td>
<td>336</td>
<td>10800,00</td>
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<td></td>
<td>672</td>
<td>16950,00</td>
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</table>

*Significant difference, with a confidence level of 5%.
It was possible to recover and identify *C. pseudotuberculosis* from the pus of animal 66 seven (7) days after the inoculation, from the milk secretion of animal 117 forty eight (48) hours PI and from the supra mammary lymph node and mammary gland of animals 119 and 117 respectively at the time of slaughtering.

All the animals showed the supra mammary lymph nodes enlarged, but without abscesses. Visible macroscopic alterations were not seen in the organs examined, except a gelatinous edema at the sites of inoculation. Animal 117 had a large quantity of caseous secretion in the udder above the cut.

**DISCUSSION**

**ETREBY & ABDEL-HAMID** (1970) performed an experimental infection via galactofer canal in healthy sheep with *C. ovis* and *C. pyogenes* and observed the development of chronic purulent catarrhal acute mastitis and chronic apostematous mastitis, respectively.

The microorganism in the mammary parenchyma demands chemotherapeutic action for neutrophil and macrophages that perform phagocytosis and constitute the initial focus of abscess formation that expand through the secretory tissue (ETREBY; ABDEL-HAMID, 1970).

Even though the via of inoculation in this study was different, the formation of pustules at the site of inoculation was observed in all animals. One animal from GII had a unilateral abscess of approximately 5 cm in diameter and with a purulent content in the parenchyma of the gland. Also in this animal there was a progressive increase of mammary gland cells at different times of the EC examination. There was a predominance of whole and some degenerated neutrophils, characteristic of acute inflammatory process (COWELL; TYLER, 2002). Other GI animals had also significant increase of cells, with neutrophils predominance. Similar findings were observed by BURREL (1978) in his study with experimental inoculation of caseous lymphadenitis in sheep.

The dose of *C. pseudotuberculosis* did not cause suppurative and/ or systemic caseous lesions by the used inoculation via for GI and GII animals, even though there was some alteration in the general health status of the GII animals.

According to AUGUSTINE (1982) the concentration of viable organisms in the purulent material is estimated to be from $1 \times 10^5$ to $5 \times 10^7$ per gram of pus to cause the disease in animals. Other aspect that must be considered is the period of observation that usually
is not long enough for the lesions formation in the internal lymph nodes and other organs as was described by Langenegger, Langenegger (1988) that kept goats under observation for 126 days. Johnson et al. (1993) intra dermically inoculated 4 x 10^6 washed bacteria and recorded a chronic disease with the formation of internal and external abscesses without any signs of the acute phase such as fever, loss of appetite and hemolysis, the period between inoculation and abscesses rupture was 3 to 6 weeks, less than the one observed by Ashfaq; Campbell (1979), 90 days. Penn et al. (1988) carried out a subcutaneous experimental infection of C. pseudotuberculosis on sheep at different doses and observed local reaction and multiple abscesses formation in the lymph nodes 21 days after inoculation.

Nagy (1976) also carried out experimental infection using different routes of infection and showed that there exists a link between the point of inoculation and the region lymph node affected. In the present study the occurrence of lesions at the site of inoculation, the formation of edema, erythema, papules, ulcers and crust in GI animals lead to the bacteria recovery from some of these lesions. However, related to the recovery of C. pseudotuberculosis from the lymph nodes and milk secretion, it was possible to isolate the bacteria from 50% of the animals inoculated which is in accordance with the findings of Garg; Chandiramani (1985) who also did not recover the bacteria from all the animals.

The formation of edema at the inoculation site probably occurred due to the action of the toxin produced by C. pseudotuberculosis, which affected the blood vessels in vivo. Johnson et al. (1993) in their study discussed the role of this toxin in the pathogenesis of naturally acquired disease.

Gamell; Tartour (1974) analysed the hemograms and plasma protein of sheep experimentally infected with C. pseudotuberculosis, and reported a severe hemolytic anemia, described as transitory macrocitic hypochromic, as well as hypochromic normocytic anemia. This was not observed in our study.

In relation to the leucocytosis observed in the present study, Jain (1993) reported that, among other causes, bacterial infections are responsible for leucocytosis, generally related to neutrophilia. The neutrophilia is the result of increased transference of cells from the marginal pool to the circulation, reducing the migration of cells to the tissues and increasing the production and liberation of neutrophils by the bone marrow. Also according to Meyer et al. (1995), intense acute inflammation and those similarly found in GI and GII animals, neutrophilia oocurs from a stimulus and continual tissue demand, elevated production and liberation of neutrophils to suppress the tissue demand. Even with the significant difference in the evaluation of the monocytes obtained in GII during the experimental phase, no monocytosis was observed. According to Kerr (2004) monocytosis occurs just prior to chronic infections, more frequently related to parasitic or bacterial infections.

**CONCLUSION**

From the results of this experiment it was shown that C. pseudotuberculosis, isolated from a natural case of caseous lymphadenitis, could cause acute clinical mastitis. The results from the expression cytology tests were satisfactory as an auxiliary diagnostic method and could be considered for use in future studies on goats.

**REFERENCES**


J.W. Pinheiro Junior et al.


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