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Ultrasound and histopathological investigations of experimentally induced *Staphylococcus aureus* mastitis in goats

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ABSTRACT

The purpose of the present study was to investigate the ultrasound and histopathological findings in experimentally induced *Staphylococcus aureus* mastitis in goats. The experiment was conducted using six clinically healthy goats, 2–4 years of age, weighing 48–54 kg. The experimental infection was done with stationary-phase culture of a field *S. aureus* strain isolated from the milk of a cow with clinical mastitis. Ultrasound measurement of udder structures established statistically significant changes in teat canal length, teat wall thickness and diameter of lactiferous ducts. A clear sign of inflammation was the ultrasound visualization of hyperechoic structures in the teat cistern, which were identified as milk coagula after milking. The ultrasonography of udder parenchyma on post infection hour 72 showed large hyperechoic zones, statistically significant ($P < 0.05$) narrowing of lactiferous ducts and inability for visualization of anechoic blood vessels. On post infection hour 168 that corresponded to the 3rd day of treatment of goats, teat cistern content was normal and anechoic. Histopathological results showed vacuolar degeneration of epithelial cells, serous exudate in glandular duct lumens consisting of multiple neutrophils, as well as interstitial changes–haemorrhages, oedema and mononuclear proliferations of lymphocytes and histiocytes. The analysis of results indicates that ultrasonography combined with histopathological examinations could be successfully used for detection and monitoring of the time course of goat udder alterations caused by experimental *S. aureus* mastitis.

1. Introduction

According to the available literature, experimentally induced clinical or subclinical mastitis has been mainly reported in cows. During the last decade, a number of studies have investigated the experimental infection of these animals with *Staphylococcus aureus*. They have used various infection doses depending on the research goal (van Herwijnen, 2010). In goats, clinical *S. aureus* mastitis was reproduced with doses of 18×10^3 (Rainard, 2007), 1×10^4 and 1×10^8 cfu (Ma et al., 2007). Lasagno et al. (2012) have followed the course of experimentally induced subclinical mastitis in goats, inoculated with *Streptococcus uberis* at a dose of 1.7×10^8 cfu. A number of

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studies have investigated the experimental induction of Mycoplasma mastitis in cows, sheep and goats (Rana et al., 1992; Sanchis et al., 2000; Byrne et al., 2005; Castro-Alonso et al., 2009). Jing et al. (2012) evaluated the time course of interleukin-17 (IL-17) associated cytokines in experimentally induced *Escherichia coli* mastitis in goats. Thoria et al. (2011) reported the effect of *Terminalia brownii* methanolic extract in goats with experimental *S. aureus* mastitis.

Ultrasound findings of inflamed udder have been described in cows (Banting, 1998; Flöck and Winter, 2006; Franz et al., 2009; Javadi and Acorda, 2011). Ultrasonography of the mammary gland in goats is performed primarily to measure the teat cistern and the teat canal, in order to establish the changes occurring after machine milking (Salama et al., 2004; Wójtowski et al., 2006; Ślósarz et al., 2010).

To our best knowledge, there are no reports about ultrasound and histopathological changes occurring in goat's udder with experimentally induced staphylococcal mastitis.

The aim of the present study was to establish the ultrasound and histopathological findings in goats with experimental *S. aureus* mastitis.

2. Materials and methods

2.1. Experimental animals

The experiment was approved by the Animal Ethics Committee to the Faculty of Veterinary Medicine, Trakia University–Stara Zagora, in compliance to Ordinance No. 20 of the Ministry of Agriculture and Food from 1 November 2012 on the minimum requirements for the protection and welfare of experimental animals and the requirements for the utilization, rearing and/or delivery facilities. The experiments were conducted using six clinically healthy Bulgarian native goats, 2–4 years of age, weighing 48–54 kg. The animals were reared in the bio base of the Faculty of Veterinary Medicine–Stara Zagora. The goats were in the 6th lactation month, first to third lactation, and were milked manually in the morning and in the evening.

2.2. Clinical examination

Prior to the experiment's start (hour 0), rectal body temperature, heart and respiratory rates, colour of visible mucous coats and rumen movements were recorded. The clinical examination of the udder included inspection, palpation and California mastitis test of the milk. All aforementioned parameters were monitored on hour 0 and post infection hours 6, 24, 48, 72, 96, 144 and 168.

2.3. Udder ultrasonography

Ultrasonography was performed with SonoScape A5v (SonoScape, China) unit equipped with linear multifrequency transducer (5.0–12.0 MHz). The transcutaneous scans were performed using vertical (for teat) and horizontal (for udder parenchyma) position of the transducer and frequency 12.0 MHz. For better contact between the transducer and udder skin, ultrasonography gel (Eco-Ultra gel, Milano, Italy) was used. Initially, the possibility for visualization of teat and udder parenchyma structures was evaluated and thereafter, the following parameters were measured: teat canal length, teat canal diameter, teat cistern diameter in the area of the rosette of Furstenberg, in the middle part of the cistern and in the area of transition between teat and gland cisterns. Ultrasound measurements were conducted and calculated by the software of the ultrasound machine. The echogenicity of parenchyma, the imaging of parenchymal lactiferous ducts and blood vessels, and the presence of milk coagula in the teat were all assessed. These parameters were monitored on hour 0 and post infection hours 24, 48, 72, 96, 144 and 168.

2.4. Bacteriological study and treatment

In this experiment, a stationary-phase culture of a field *S. aureus* strain obtained from a cow with clinical mastitis was used. The infection dose was standardized using the first tube of McFarland's standard–0.5/or 1.5×10^8 cfu/ml. The staphylococcal strain was cultivated on blood agar (Trypticase-soy agar base, Difco, supplemented with 7% sheep erythrocytes) at 37 °C and the stationary growth phase was used for preparation of bacterial culture for inoculation. The bacterial culture was administered intracisternally at a dose of 1 ml in one half of udders. The other udder halves served as negative control and they were treated with 1 ml of saline solution. Microbiological monitoring of experimental infection was done on hour 0 and post infection hours 6, 24, 48, 72, 96, 144 and 168. Milk samples were collected from infected and control udder halves of all goats, which were bacteriologically examined for presence of *S. aureus*. After the performed antibiogram testing of the used staphylococcal strain, the goats were treated with antibiotics on post infection hour 96 (Tetracycline hydrochloridum 200 mg; Neomycin sulphate 250 mg; Bacitracin 2000 UI; Prednisolone 10 mg), using an intramammary injector (Mastijet fort, MSD, Holland), applied intracisternally, three times at 12-hour intervals.

2.5. Histopathological study

Immediately before the first antibiotic application (post infection hour 96), incision biopsy of the udder was performed (Knight et al., 1992). Five ml Novocain 1% (Vetprom, Radomir, Bulgaria) were infiltrated along the incision cut and then, another 6 ml were distributed in three points at 2 cm depth to outline a pyramid-shaped desensitized biopsy area. After aseptic preparation of the field, a 2-cm linear cut of the skin was performed, retracted using a retractor and then, a cubic specimen of approximately 1 g weight was incised with a scalpel. The cut surfaces were closed with interrupted horizontal U-shaped polyglactin 9/0 (DemeCRYL, Demetech, USA) USP 1 sutures. Skin closure was done with interrupted simple polyamide (Polikon, Tonzos, Bulgaria) USP 0 sutures.

The specimens for histological examination were fixed in 10% neutral formalin. Samples were cut on a paraffin microtome with 4 μm thickness. The cross sections were processed using the standard histological techniques (Diakov et al., 1989) and stained with haematoxylin/eosin.

2.6. Statistical analysis

Results were processed by StatSoft statistical software (Statistica 7, Microsoft Corp. 1984-2000 Inc.) using ANOVA and non-parametric comparison of proportions according to the Student's t criterion. The results were presented as means and standard deviations (Mean ± SD). Differences vs hour 0 (before infection) were considered statistically significant at $P \leq 0.05$.

3. Results

3.1. Clinical data

Only one goat (No. 3) exhibited hyperthermia (41.1 °C) on hour 48 after the inoculum application. The subsequent measurements of rectal temperature were within the normal reference range. Heart rate was slightly accelerated in goats No. 1, 3 and 5 as early as post infection hour 6. By post infection hour 24, it did not show any deviations from the reference values. During the next periods, only sporadic tachycardia (110 min^{-1}) was noticed; it was most pronounced in goat No. 2 on hour 144. Six hours after the infection, respiratory rates were higher only in two of goats. A slightly accelerated respiratory rate (36 min^{-1}) was observed in goat No. 3 by the 48th hour and in goat No. 1 (38 min^{-1}) on the 144th hour. The visible mucosae and rumen movements (11 ± 2) were normal in all goats. Clinical examination of the udder in all goats showed that