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Is udder ultrasonography a diagnostic tool for subclinical mastitis in sheep?

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ABSTRACT

This study was designed to assess the application of ultrasonography for diagnosis of subclinical mastitis in sheep. A total of 56 udder halves and their corresponding superficial inguinal lymph nodes were examined ultrasonographically using a 7.5 MHz linear transducer. Milk samples were collected for bacteriological examination, somatic cell count (SCC) and the California mastitis test (CMT). Based on the bacterial culture growth, animals were classified into 4 groups as no growth (control, $n = 4$), coagulase-negative staphylococci (CNS, $n = 14$), *Staphylococcus aureus* (*Staph. aureus*, $n = 4$), and mixed CNS and *Staph. aureus* ($n = 6$). Blood analysis results showed non-significant difference ($P > 0.05$). The echogenicity of the infected udders' parenchyma was homogenous and hypoechoic. The gland cisterns were anechoic, while the teat canal appeared as a longitudinal echogenic line. The superficial inguinal lymph nodes were easily identified and appeared as oval-shaped structure with thin echogenic capsule. All nodes had an anechoic or hypoechoic parenchyma with a linear echogenic structure at the center. The ultrasonographic length, depth and area of the superficial inguinal lymph nodes were significantly increased in the udder infected groups ($P < 0.05$). SCC values showed significant difference among different udder health groups ($P < 0.05$). SCC was correlated with the ultrasonographic length ($r = 0.59$, $P < 0.01$), depth ($r = 0.64$, $P < 0.01$) and area ($r = 0.66$, $P < 0.01$) of superficial inguinal lymph nodes. The critical threshold for ultrasonographic length, depth and area of superficial inguinal lymph node was 11.5 mm, 7.8 mm and 13.5 mm^2, respectively. Furthermore, the critical value for SCC was $\geq 400 \times 10^3$ cells/mL. In conclusion, the ultrasonography is a fast and field test for supporting diagnosis of subclinical mastitis. In addition, ultrasonographic examination of udder especially superficial inguinal lymph nodes can greatly aid in the assessment of subclinical mastitis in ewes and should be applied alongside SCC and CMT.

1. Introduction

Subclinical mastitis means inflammation of the mammary gland that does not create visible changes in the milk or the udder (Anderson et al., 2002). It causes economic losses in dairy industry. Subclinically infected sheep will produce less milk, and the quality of the milk will be changed (Leitner et al., 2008) with increased culling rate and cost of veterinary treatment (Serrano et al., 2003). In addition, infected sheep can be a source of infection to other animals in the flock (Kiossis et al., 2007).

Since there are no visible abnormalities in the milk, subclinical mastitis requires special diagnostic tests for

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detection. The California mastitis test (CMT) is a common indirect field test for measuring somatic cell count (SCC), but some authors claim that CMT is an unreliable method for diagnosing intramammary infection (IMI) (Bergonier et al., 2003; Schaeren and Maurer, 2006). In contrast, other studies report that CMT may be helpful for detection of IMI (Karzis et al., 2007; Petzer et al., 2008). SCC is a commonly used indicator of udder health in cow, sheep and goat milk (Radostits et al., 2007). Although elevated SCC is mainly a response to infection, it has certain limitations that may affect result interpretations. In ewes, somatic cell counts are higher during the first few weeks of lactation and decrease at the maximum milk production (Lafi et al., 1998). Furthermore, average SCC may be affected by parity (Rota et al., 1993) as positive relationship was noticed between SCC and parity (Králíčková et al., 2012).

Diagnostic ultrasonography is commonly used in human medicine for examination of mammary gland alongside mammogram (Gooding et al., 2010). Ultrasonography has been conducted in many studies related to the mammary gland (Wójtowski et al., 2006; Ślósarz et al., 2010). Ultrasonography is useful in describing the normal morphological appearance of sheep udder and teats (Ruberte et al., 1994; Rovai et al., 2008). Furthermore, it is diagnostic for detection and monitoring the pathological changes in the teats (Mavrogianni et al., 2004) and mammary gland (Flöck and Winter, 2006), others used ultrasonography for differentiation of structures identified in udder parenchyma such as haematoma and abscess in cattle (Lazaridis et al., 2012), and various pathological changes in caprine udder (Fasulkov et al., 2014).

Few studies have reported the diagnostic application of udder ultrasonography in ewes. In comparison with acute mastitis, subclinical mastitis is difficult to diagnose. Therefore, the objective of the present study was to investigate the use of ultrasonography in diagnosis of such problem.

2. Materials and methods

2.1. Animals and clinical examination

Twenty-eight lactating ewes of Ossimi breed were included, the average age was 3.3 ± 0.5 years and weight was 48 ± 2.3 kg (mean ± standard deviation). All animals raised at a private flock in a region of Assiut governorate, Egypt. The size of the flock was 64 sheep. The average lactation period of animals was 8–10 weeks at sampling. This study was conducted during the period from February to April 2014. The ewes were housed in a free stall barn. Animals had free access to water all the day and were fed twice daily at 8:00 h and at 17:00 h. The diet consisted of Egyptian clover (*Trichofolium alexandrium* – Barseem), wheat straw and concentrate feed mixture. Only clinically healthy ewes without obvious changes in udder or milk were included in the current study. All animals were subjected to physical examination as described elsewhere (Pugh, 2002). The clinical examination of the mammary gland included inspection and palpation of the teats, the udder and the superficial inguinal lymph nodes (Baumgartner, 1999). Furthermore, the general behaviour and condition; auscultation of the heart, lungs, rumen and intestine; and measurement of heart rate, respiratory rate and rectal temperature were also carried out. Animals with any disease condition were excluded from the study.

2.2. Udder ultrasonography

Ultrasonographic imaging of the teats, udder parenchyma and superficial inguinal lymph nodes were carried out in all ewes in the standing position using a portable Beta-mode ultrasound generator with a 7.5 MHz linear transducer (Tringa Liner, Esaote Europe B.V., The Netherlands) and a 4 MHz convex transducer (FF Sonic, UF-4000, Fukuda Denshi Co., Ltd, Japan). In preparation for ultrasonography and in order to ensure good adherence of the probe to the udder, the area of the udder skin was clipped and cleaned with alcohol before application of gel. Teat sonograms were obtained after direct contact of the transducer on the teat (Franz et al., 2001; Mavrogianni et al., 2004). Ultrasonographic imaging of udder parenchyma was carried out by direct contact of the probe on the udder skin. To scan the entire udder, the probe was placed on the caudal surface of each half along its longitudinal axis and moved upwards and downwards (Flöck and Winter, 2006). Teat and mammary gland sonograms were obtained in the vertical and the horizontal plane. The sonographic examination of the superficial inguinal lymph nodes was performed after placing the probe on the area dorsal and lateral to the caudal aspect of udder halves. The lymph node was identified as it had well demarcated echogenic capsule with an echogenic linear structure running longitudinal through the center of the node (Kofler et al., 1998). The length, the depth and the area of the node was measured as reported elsewhere (Bradley et al., 2001). At first the measurements were repeated twice to check for consistency, but as the differences were small (less than 5%), only one measurement was recorded for each ewe.

2.3. Blood sampling

Two blood samples were collected by puncture of the jugular vein: one with heparin and the other without anticoagulant. Blood gas analysis and a complete blood count including erythrocyte count, hematocrit, hemoglobin and total leucocyte count were carried out on the first sample (Coles, 1986). The blood samples were analyzed for pH, bicarbonate (HCO_3), partial tension of carbon dioxide (pCO_2), partial tension of oxygen (pO_2), and base excess (BE) using blood gas analyzer (ABL 5, Radiometer, Copenhagen, Denmark). After centrifugation of the second blood sample, serum samples were collected and then frozen at −20 °C for one week; subsequently, analysis of biochemical parameters was carried out. With the serum samples, commercial test kits were used to determine the concentrations of total proteins, albumin, blood urea nitrogen, creatinine and total bilirubin. The activities of aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (GGT) were also measured in serum samples. The biochemical analyses of the selected parameters were spectrophotometrically measured according to the standard protocols of the suppliers.

2.4. Milk sampling, bacteriological examination and determination of SCC and CMT

Before milk sampling, the teat ends were cleaned by 70% alcohol. The first strips of milk were discarded, and then 10 mL of milk was collected aseptically in sterile tubes. Samples were refrigerated in an ice box and transported immediately to the laboratory. Ten microliters of each milk sample was plated on blood agar (HIMEDIA, India), containing 5% of sheep blood. The plates were incubated aerobically at 37 °C for 24 h. The suspected colonies were identified morphologically by Gram's stain and biochemically confirmed as described previously (Quinn et al., 1999), using catalase and coagulase tests. *Staphylococcus aureus* (*Staph. aureus*) was identified by means of typical colony morphology and coagulase reaction (coagulase-positive) when typical hemolysis zones were not present. Coagulase-negative staphylococci (CNS) were identified by typical colony morphology and negative coagulase reaction.

Based on the degree of viscosity, milk samples were tested by CMT and categorized into five scores, where 0, no viscosity; T, trace slime; 1, slime without gel; 2, obvious gel formation; 3, gel agglutinated to the bottom of the plate (McDougall et al., 2001; Fragkou et al., 2007). To improve the reliability of the evaluation, CMT scores were carried out by the same person. For each milk sample, the SCC was determined by direct microscopic cell counting method using methylene blue staining (Petersson et al., 2011).

2.5. Statistical analyses

Data were analyzed statistically using SPSS software (SPSS analytical program for Windows Version 20; SPSS GmbH, Munich, Germany). The normal distribution of all variables was tested using Kolmogorov-Smirnov normality test. All parameters were normally distributed. Differences between non-infected and infected groups were assessed by one-way analysis of variance with post hoc Bonferroni multiple comparison test. Pearson's correlation procedure was used to determine the relationship