



Prevalence of *Staphylococcus aureus* subsp. *anaerobius* in Sub-Clinical Abscess Cases of Sheep

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Short communication

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ABSTRACT

Aim: To investigate the prevalence of *Staphylococcus aureus* subsp. *anaerobius* (Saan) in sub-clinical lymph node abscesses (SLNA) in sheep.

Place and Duration of Study: Department of Microbiology (Faculty of Veterinary Medicine) and Unit Microbiology and Molecular Biology (Institute for Studies and Promotion of Animal Exports), between May 2003 and Dec. 2005.

Methodology: Enlarged superficial lymph nodes (n=137) were taken from sheep carcasses at meat inspection and were subjected for bacteriological examination.

Results: Pure cultures of Saan were obtained from 44% of the pus samples, *Corynebacterium* spp. from 33% and *S. aureus* subsp. *anaerobius* from 7%. The rest of the pus samples yielded mixed cultures of Saan with either *Macrococcus caseolyticus* (10%) or *Corynebacterium* spp. (6%).

Conclusion: Although these results show *S. aureus* subsp. *anaerobius* as the prime cause of SLNA, they also show the importance of inclusion of *Corynebacterium pseudotuberculosis* in vaccine developments or vaccination protocols against abscess

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diseases of sheep, especially those intended for export.

Keywords: *Staphylococcus aureus* subsp. *anaerobius*; *Corynebacterium* spp.; *Macrocooccus caseolyticus*; sheep abscess.

1. INTRODUCTION

Abscess syndrome or Morel's disease is an economically important, non-fatal, contagious disease that affects sheep and goats (Bajmócy et al., 1984; El Sanousi et al., 1989; Alhendi et al., 1993; Szalus-Jordanow et al., 2011). It is caused by *Staphylococcus aureus* subsp. *anaerobius* - shortly referred to as *S. aureus anaerobius* - (de la Fuente et al., 1985; Musa et al., 2011). It is normally encountered in lambs between 4 and 10 months of age and is characterized by the formation of abscesses in or adjacent to superficial lymph nodes (parotid, prescapular, prefemoral, etc.). This disease is considered as one of the major disease constraints of Sudan's sheep exports (Freigoun et al., 2009). Many Sudanese shipments of export sheep were rejected by Saudi Arabia on the grounds of this disease, the economic losses of which amounted to some millions of US dollars. Sub-clinical form of abscess disease is presented as enlargements of superficial lymph nodes that are detected during meat inspection, causing condemnation of carcasses, with consequent great economic losses, especially for export meat industry. Morel's disease is sometimes misdiagnosed as caseous lymphadenitis or pseudotuberculosis (caused by *Corynebacterium pseudotuberculosis*). Both diseases share the same clinical picture of formation of superficial lymph node abscesses. Differentiation between the two diseases is based on many factors including the age of the animal in addition to microscopic and bacteriological examination of the pus material. In the present investigation we show that both *S. aureus anaerobius* and *Corynebacterium* spp. are important in causing sub-clinical abscesses of sheep in the Sudan.

2. MATERIALS AND METHODS

2.1 Samples

One hundred and thirty seven enlarged superficial lymph nodes (approximately 3x4 cm in diameter) were collected from carcasses of sheep at meat inspection in three different slaughter houses in Khartoum State. Intact lymph nodes were incised from the surrounding tissues and transferred to the lab in thermo-flasks containing ice. Pus samples were collected from lymph nodes as follows: lymph nodes surfaces were disinfected with pieces of cotton soaked in 70% alcohol followed by the application of hot spatula, small incisions were made using sterile blades through which the pus was squeezed near the flame into sterile universal bottles and stored at - 20°C until being cultured.

Direct smears were prepared from the fresh pus samples and stained with Gram's stain. For cultures, loopful pus samples were streaked on 10% sheep blood agar plates and incubated in candle jars at 37°C for 48 hr.

2.2 Identification of Bacteria

2.2.1 Conventional bacteriology

Isolated bacteria were subjected to bacteriological tests according to Barrow and Feltham (1993). Identification of staphylococci was based on the second edition of Bergy's manual of systematic bacteriology (Sneath et al., 1986). Isolates were tested for coagulase, sensitivity to, novobiocin, aerobic and anaerobic growth, catalase, oxidase, urease, nitrate, blood agar haemolysis, VP, pigment production, colony size, in addition to fermentation of different sugars.

2.2.2 PCR

Twenty one of the isolates conventionally identified as *S. aureus anaerobius* in addition to two reference strains of this organism were subjected to PCR amplification of the *nuc* and *kat* genes. Genomic DNA was extracted using Axy Prep Bacterial Genomic DNA Miniprep Kit of Axygen (Bioron, Ludwigshafen, Germany) with some modifications of the manufacturer's protocol. In brief, 3–5 colonies of *S. aureus anaerobius* obtained from 48 hr blood agar cultures were suspended in 150 µl of the recommended buffer (after addition of the RNase); 10 µl of Lysostaphin (Sigma, Taufkirchen, Germany) 1 mg/ml were added to the mixture and incubated at 37°C for 1 hr; 2 µl of 10% Proteinase K (Bioron) was added and incubated at 56°C for 2 hr. For the detection of the *nuc* and *kat* genes, primers (*nuc*1: 5' GCGATTGATGGTGATACGGTT 3', *nuc*2: 5'AGCCAAGCCTTGACGAACTAAAGC 3', *cat*808F: 5' CTCCATTTTAGAACGCAACAA 3', *cat*1583R: 5' TGGGTCAGCTTTGTAACA 3') and conditions mentioned by Brakstad et al. (1992) and Sanz et al. (2000), respectively, were used.

3. RESULTS

Out of 137 enlarged lymph nodes *Staphylococcus aureus* subsp. *anaerobius* (Saan) was isolated in pure cultures from 60 pus samples (43.79%) and from mixed cultures of 22 samples (16.05%). In 14 samples (10.21%), Saan was mixed with *Macrococcus caseolyticus* and in 8 samples (5.83%) it was mixed with *Corynebacterium* spp. Other bacteria isolated in pure cultures were *Corynebacterium* spp. and *S. aureus* subsp. *aureus* in frequency rates of 32.84% (45 samples) and 7.29% (10 samples), respectively. Primers for *nuc* and *kat* genes gave positive amplifications results (amplicons of about 270 bp and 775 bp, respectively) in PCR for all of the tested *S. aureus anaerobius* isolates.

4. DISCUSSION

This study was designed to investigate for the prevalence of *S. aureus anaerobius* in superficial lymph node abscesses in slaughtered sheep and to identify candidate staphylococci that can be associated with abscess disease of sheep. Enlarged superficial lymph nodes (prescapular and popliteal) are routinely palpated/ incised by the veterinarians at meat inspection of the carcass. These lymph nodes together with the parotid lymph node were found to be the mostly affected lymph nodes in sheep with abscess syndrome or Morel's disease (Hamad et al., 1992). Here, we use the term "sub-clinical abscess disease" when enlargement of these lymph nodes is only detected at carcass examination. This would rather differentiate the condition of animals with invisible enlarged superficial lymph nodes from those with visible ones, which cause rejection of animals during antemortem

examination. Although identification of bacteria in this study was made based on conventional biochemical tests, at least identification of *S. aureus anaerobius* was confirmed by PCR to the species level. Moreover, complete or partial sequencing of the catalase gene of 9 *S. aureus anaerobius* strains {please see supplementary materials link} isolated in this study confirmed the identification to the subspecies level (Musa et al., 2010). In this study *S. aureus* bacteria (*S. aureus anaerobius* and *S. aureus* subsp. *aureus*) were found the mostly prevalent bacteria in sub-clinical abscess disease of sheep. *S. aureus anaerobius* causes sheep abscess (or Morel's) disease, which normally affects sheep at young ages. So, higher prevalence of *S. aureus anaerobius* in sub-clinical abscess is expected in sheep intended for export, because these animals are normally slaughtered at younger ages due to consumer preferences in importing Gulf countries. In addition, export sheep are usually subjected to fattening process, which has been found to trigger abscess formation (Babiker and El Sanousi, 2004). *Corynebacterium pseudotuberculosis* causes caseous lymphadenitis, which normally affects older animals. It is interesting that both *S. aureus anaerobius* and *C. pseudotuberculosis* were isolated in this study from the same lymph node abscesses of 8 animals. While *S. aureus anaerobius* requires microaerophilic or anaerobic conditions for growth, *Corynebacterium* spp. is facultative anaerobic. So, which one has favoured the conditions for the other requires further investigations. This might withdraw the attention to the possibility of both organisms being present in clinical cases of the disease. However, in an outbreak of abscess disease in a flock of sheep (Musa et al., 2007), 28 out of 100 animals were infected with *S. aureus anaerobius*, only two animals were infected with *Corynebacterium* spp. and no mixed infection was found. *Macrococcus caseolyticus* is a member of the Staphylococcaceae family and it was classified as *Staphylococcus caseolyticus* before being transferred to the new genus *Macrococcus* (Kloos et al. 1998). It can be isolated from milk and meat products of sheep, goats and cattle. This organism was isolated in association with *S. aureus anaerobius* from about 10% of the enlarged lymph nodes of sheep in the present study. De la Fuente et al. (1992) made the first report on the isolation of this organism from abscesses of slaughtered lambs – also in association with *S. aureus anaerobius*, but to the best of our knowledge, no further reports were made on the isolation of this organism from diseased animals. Significance of the association of this organism with *S. aureus anaerobius* in abscess disease requires further investigation.

5. CONCLUSION

In conclusion, results of this study show that *S. aureus anaerobius* is the prime cause of superficial lymph node abscesses of sheep. However, isolation of *Corynebacterium* spp. from more than one third of sub-clinical abscesses necessitates inclusion of this organism in vaccine developments and/ or vaccination protocols against abscess disease for sheep intended for export from the Sudan.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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