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AN OUTBREAK OF ABSCESSES DISEASE IN A SHEEP FLOCK AT ISMAILIA GOVERNORATE EGYPT (With One Table and One Figure)

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وباء الأصابة بمرض الخرايج في قطعان أغنام بمحافظة الإسماعيلية-مصر

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ظهر وباء مرض الخراجات بمزرعة اغنام بمحافظة الاسماعيلية سعتها ٧٥٠ من الأغنام المحلية وكانت نسبة الاصابة ١٢٠ من ٢٠٠ (٦٠%) فى الاغنام صغيرة السن (٦-١٠ شهور). ولم يكن هناك اى ظهور للمرض فى الاغنام البالغة. وقد ظهر المرض لأول مرة بعد مرور ثلاث أسابيع من جز الصوف وكانت نسبة الخراجات عالية في مناطق الرأس والعنق والكتف بالقرب من الغدد الليمفاوية وقد أثبت الفحص البكتريولوجي عزل ميكروب البكتيريا العنقودية العنزة اللاهوائية من الخرايج الموجودة تحت الجلد والتي تحتوي على صديد أبيض مصفر لزج عديم الرائحة وقد تم دراسة الصفات الكيميائية للعترات المعزولة وأثبتت الدراسة أن جرثومة البكتيريا العنقودية العنزة اللاهوائية لها حساسية ضد المضادات الحيوية التالية: الأمبسلين والاموكساسيلين والكلوكساسيلين والجنتاميسين والأوكسى تتراسيكلين والأستربتوميسين ومقاومة للبنسيلين والإرثرومييسين وقد تم العلاج عن طريق التدخل الجراحي واستخدام المضاد الحيوى بن-سترب المكون من ٢٠٠٠٠٠٠ وحدة دولية بروكاين بنسلين و ٢٠٠ مجم ستربتوميسين بجرعة ٥مل يوميا لمدة أربعة أيام حقن بالعضل للأغنام المصابة والمعرضة للإصابة وقد كانت استجابة الأغنام للعلاج جيدة وقد تم تنفيذ برنامج صارم للسيطرة على المرض وبالخصوص أثناء عملية جز الصوف وذلك لإحتمال أن هذا المرض قد أنتشر أثناء عملية جز الصوف وعليه تعتبر عملية جز الصوف من العوامل التي تساعد على انتشار هذا الوباء.

SUMMARY

An outbreak of abscess disease occurred in a 750 native sheep flock located in Ismailia governorate (Egypt). 120 out of the 200 animals (60%) of the young sheep (6-10 months old) were affected by the disease. No abscesses were detected in the adult sheep. Clinically

diseased cases were first detected three weeks after shearing. Abscesses were mostly located in the head, neck and shoulder regions close to the regional lymph nodes. *Staphylococcus aureus subsp. anaerobius* was isolated in a pure form from animals infected with subcutaneously located abscesses containing viscous white-yellow odorless pus. The biochemical characters were studied. Susceptibility testing was performed by the agar-disk diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS). The isolates were resistant to penicillin and erythromycin and sensitive to, ampicillin, amoxicillin, cloxacillin, gentamicin, streptomycin and oxytetracyclin. Surgical therapy remedy and intramuscularly pen-strep® antibiotic injection (Procaine penicillin G 200,000 Iu. + Dihydro streptomycin Sulphate 200mg) was started once daily and continued for four days. The affected sheep responded well to the treatment, with complete resolution of the abscess. A firm control program was applied especially during shearing cause it was highly probable that the animals in the present outbreak became infected about shearing time. Therefore, shearing was the risk factor most probably associated with the outbreak.

Key words: *Morel's abscess disease, skin infection, sheep, Staph aureus.*

INTRODUCTION

Morel's abscess disease is a specific skin infection of sheep and goats, which affects mainly young animals up to about 6 months of age, and characterized by the presence of abscesses in subcutaneous tissue. The abscesses are the only obvious manifestation of the disease, and they are most frequently located next to the lymph nodes of the mandibular region (mandibular, parotid and lateral retropharyngeal), followed by the superficial cervical, subiliac and popliteal nodes in that order of frequency (De la Fuente and Suarez, 1985 a; De la Fuente *et al.*, 1985b).

The organism causing abscess disease is identified as a respiratory deficient variant of *Staphylococcus aureus* and taxonomically named (*S. aureus subsp. Anaerobius*) (De la Fuente and Suarez, 1985a; De la Fuente *et al.*, 1985c). Abscess disease in sheep has been repeatedly diagnosed in several countries, mainly France (Aynaud, 1928; Boukerrou *et al.*, 1985) and Spain (De la Fuente and Suarez, 1985a; De la Fuente *et al.*, 1985b). Two outbreaks have been described in goats in Italy (Valenti and Bieler, 1984) and Sudan (El Sanousi *et al.*, 1989).

Abscess disease causes economic losses because of a reduced growth rate of the affected animals, condemnations in abattoirs and reduced the international trade with live animals. The abscess disease of sheep and goat can be differentiated from caseous lymphadenitis (CLA), which affects the adult animals and is characterized by abscesses in superficial and visceral lymph nodes, (Brown and Olander, 1987).

An outbreak of cutaneous abscessation in a commercial sheep flock at Ismailia governorate, Egypt is occurred (June, 2006). Clinical and epidemiological descriptions of the occurred outbreak were aimed in the present work.

MATERIALS and METHODS

Case history: The disease occurred in a commercial Baladi sheep flock (n=750 heads). The owner reported that the disease was first noticed about 3 months earlier after the wool had been shorn and the flock had not a previous history of abscess disease. The flock was manually shorn at beginning of June. After shearing for several days, the sheep were kept under cover for about 2 h and a shower dip was not used. Clinical cases were first recognized by the farmer 3 weeks after shearing. Clinical examination of the respective flock was conducted, and revealed that 120 out of 200 young sheep group (60%) showed abscesses next to one or more superficial lymph nodes. Age of the diseases cases ranged from 6 to 10 months old and no abscesses were detected in yearling and adult sheep. The young sheep (either male or female) group had been housed apart from adult ewes and was mixed for shearing and had shorn after the adults. The farmer claimed that at Shearing time, three ewes, about 20 months of age, showed an abscess, which unhygienically excised by the shearers.

Sampling for bacterial culture: Using a sterile syringe, about 1 ml of pus was withdrawn from subcutaneously located abscesses (n=22 diseased sheep) for bacteriological examinations, animal's representative of the different stages in the development of the abscesses and different locations were selected. Pus smears were stained by Gram's stain. Each sample was cultured on three sheep blood agar plates, which were incubated at 37°C, one aerobically, one microaerophilically (10% carbon dioxide) and third one anaerobically (10% H₂, 10%CO₂, and 80% N₂). Cultures were examined daily for five days and isolates were identified and stored on agar slopes at 4°C.

Identification of the isolates was carried out as described by (De la Fuente *et al.*, 1985c).

Sensitivity testing: Susceptibility testing was performed by the agar-disk diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS, 2002) performance standard under anaerobic condition. The used antibiotic discs were ampicillin (10ug), amoxicillin (25ug), cloxacillin (5ug), gentamicin (10ug), penicillin G (10u), streptomycine (25ug), erythromycin (30ug) and oxytetracycline (30ug) that were supplied from Oxoid (Cambridge, CB5 8BZ, UK) and commonly used in treatment of staphylococcal infection. The clearance and diameter of inhibition zones were measured for each plate and isolates were categorized as sensitive and resistant.

Control: Control measures were performed by isolation of all affected cases that were surgically treated under a septic condition. The abscesses were excised, cleaned thoroughly, washed with H₂O₂ 2%, filled with a drain soaked with pen-strep® antibiotic, and drained daily plus a complete course of antibiotic. All susceptible cases were injected intramuscularly with pen-strep® 20/20 Inj. susp. antibiotics (Procaine penicillin G 200,000 Iu. + Dihydro streptomycin Sulphate 200mg, Intermedicavet, Animal Health Products, El-Serag Mall, Nasr City, Cairo, Egypt) once daily for four days. The hygienic measures were taken to avoid contamination of sheep skin with pus of infected cases especially during shearing. Attention was given to shears at shearing time. Good hygiene and disinfection was applied in the shearing shed, especially of the shearing board and holding pens. Younger sheep were shorn first and all sheep with palpable lesions were cleaned up first before shorn. Close contact of sheep and dipping following shearing were avoided. All wire or other causes of skin trauma in sheep housing were discarded, and moreover, purchase of animals from herds with a history of abscessation was avoided.

RESULTS

Clinical examination: One hundred and twenty sheep were found to have subcutaneous abscesses near ($n=57$) or far ($n=63$) from various superficial lymph nodes. Usually the abscesses burst within three weeks and discharged viscous creamy pus.

Clinical findings: Disregarding the abscesses, all lambs remained in good condition, there were no systemic reaction. The abscesses occurred mainly in the submandibular and cervical regions as shown in (Fig.1), in many lambs (n= 25) two or more abscesses occurred simultaneously. Likewise, several animals suffered from recurrent development of abscesses. Abscesses were rounded and gradually increasing in size up to 10 cm in diameter. The fistulated abscesses spontaneously expelling viscous white-yellow odorless pus. The number of affected animals gradually increased reach up to 60% of the animals. Attempts to stop the spread of the infection were carried out by isolation of unaffected animals in separate barns, using separate equipment in the two sections, and by thorough hand-washing. However, these attempts were unsuccessful, and the apparently healthy lambs in the clean sections developed abscesses with a similar incidence to those in the infected section. Older sheep remained clinically normal throughout the outbreak of abscess disease among the lambs. The occurrence of abscesses slowly decreased after application of firm control program.

Bacteriology: *Staphylococcus aureus subsp. anaerobius* was isolated in pure culture from all abscesses with a viscous white-yellow odorless pus. Twenty two pus smears were examined and revealed gram positive cocci that occurs in singly, in pairs and in clusters, no bacterial growth was obtained in the plates incubated aerobically, but a pure culture of pin-point, circular, low convex colonies was observed in plates incubated under microaerophilic conditions (10% CO₂) after 4-5 days of incubation. However, blood agar cultures incubated at 37°C in an atmosphere of carbon dioxide (anaerobic) yielded gram-positive cocci, after 48 hours incubation. Fully developed colonies were round, 1.0 to 1.5 mm in diameter, and surrounded by a zone of B-haemolysis. The biochemical characters of the Gram-positive cocci are shown in Table I. The identification of the isolates as *S. aureus subsp. anaerobius* was confirmed by Gram stain, catalase and coagulase tests, some subcultured isolates (n=5, 22.7%) showed bacterial growth (yellow pigmented colonies) in the plates incubated aerobically.

Control: The sheep responded well to the treatment and occurrence rate of abscess appearance were sharply decline two months post treatment and control. The flock was kept under supervision for one year; seven new cases had been appeared after collection of samples for diagnosis of brucellosis and vaccination of the flock against foot and mouth disease.

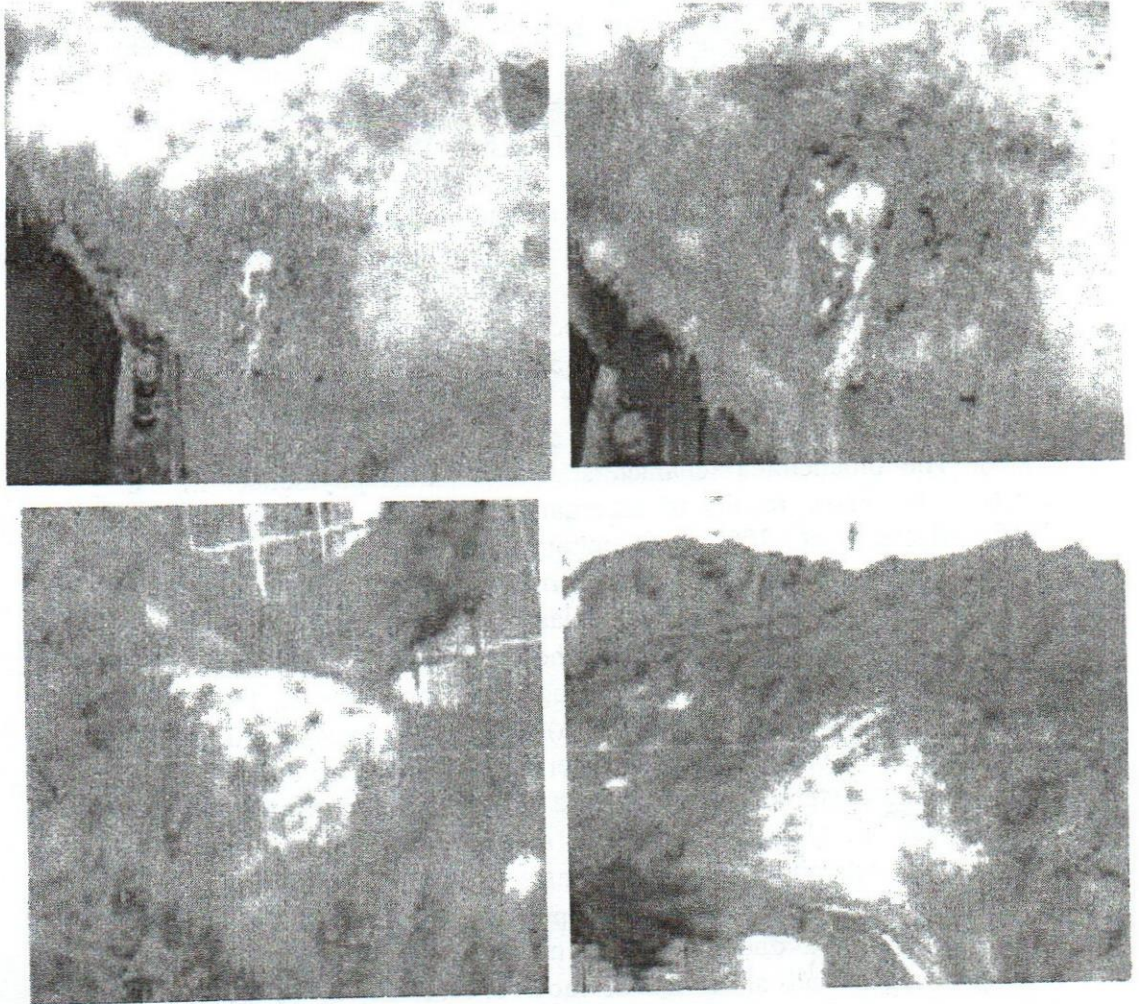
Sensitivity testing: according to clearance and inhibition zone diameter (IZD) of tested antibiotics discs against different isolates of *Staphylococcus aureus subsp. Anaerobius* {0 to 11 mm Resistance (R) more than 15 mm Sensitive (S), in case of penicillin G 0 to 20 mm Resistance (R) more than 28 mm Sensitive (S)}, the isolates were classified as resistant to penicillin and erythromycin and sensitive to ampicillin, amoxicillin, cloxacillin, gentamicin, streptomycine and oxytetracycline.

Table 1: Characterization of 22 field isolates of *Staphylococcus aureus subsp. Anaerobius*.

Characteristic	<i>S. aureus</i> ssp. <i>anaerobius</i>	<i>S. aureus</i> *
Gram staining	Gram-positive coccus	Gram-positive coccus
Aerobic growth	-	+
Micro-aerophilic	+	+
Anaerobic growth	+	+
Catalase	-	+
Coagulase	+	+
Haemolysis	+	+
Urease	-	+
Fermentation of Sucrose	+	+
Fermentation of Maltose	+	+
Fermentation of mannitol	-	+
DNASE	+	+
Benzedine	-	+
Pigment production	-	+

* Reference strain kindly supplied from microbiology dept. Vet Med, Suez Canal University.

Fig. 1: Abscess of affected sheep.



DISCUSSION

The disease described appears to be similar to "Morel's abscess disease" which has been reported in the Dauphinois breed of sheep in France, (Morel 1911), and in Kenya and Somalia (Joubert 1958). As mentioned in our result 22.7% of strains gradually acquired the cultural characters of *Staph. aureus*, the same was described by Shirlaw and Ashford (1962) who considered that the organism of abscess disease was an aberrant form of staphylococcus because after many subcultures, it gradually acquired the cultural characters of *Staph. aureus*, and they also added that some strains produced yellow pigmented colonies on subculture.

This form of staphylococcus requires carbon dioxide for normal growth, is frequently catalase negative and coagulase positive, and grow on blood agar. They appear, therefore, to be similar to the micrococci responsible for Morel's abscess disease. The bacteriological examinations of the isolated staphylococci in the current work gave results in accordance with others (Shirlaw and Ashford, 1962; Bajmo'cy *et al.*, 1984; De la Fuente and Suarez, 1985a; De la Fuente *et al.*, 1985b; Alhendi *et al.*, 1993; Møller *et al.*, 2000) and in contrast with El Sanousi *et al.* (1989) who stated that *Staphylococcus aureus subsp. anaerobius*, showed weak growth under microaerophilic (10% CO₂) condition. Species of the genus *Staphylococcus* are characterized by the production of catalase, and there are two staphylococcal species, *Staphylococcus saccharolyticus* and *Staphylococcus aureus subsp. anaerobius*, are not able to produce catalase (Kloos, and Bannerman, 1999). The biochemical characters of isolated staphylococci in the current work gave results in accordance with (De la Fuente *et al.*, 1985c; Møller *et al.*, 2000) and contrary to (Joubert 1958; Shirlaw and Ashford, 1962) who mentioned that *S. aureus subsp. anaerobius* did not able to produce hemolysins and coagulase.

The signs were restricted to the occurrence of subcutaneously located abscesses as reported from Hungary (Bajmo'cy *et al.*, 1984) and Spain (De la Fuente and Suarez, 1985a). The frequency and distribution of lesions in submandibular and cervical regions were similar to findings in other studies of abscess disease (Shirlaw and Ashford, 1962; Bajmo'cy *et al.*, 1984; De la Fuente and Suarez, 1985a; El Sanousi *et al.*, 1989; Alhendi *et al.*, 1993; Nasreen *et al.*, 2007). The lesions predominantly occurred in the prescapular region or in the head that might be explained due to wounds made by sharp object, metallic feeding troughs and shearing. In most sheep, the abscesses were definitely located in the subcutaneous tissues and did not originate from primary lymph node lesions. In some animals (n= 57), abscesses were located in close association with lymph nodes, and due to the tissue destruction associated with abscesses and the expanding nature of these, it was impossible to determine if such abscesses were of lympho nodular origin. It is most likely that abscesses due to infection with *S. aureus subsp. anaerobius* develop in the connective tissue. These findings are in accordance with observations by Bajmo'cy *et al.* (1984), El Sanousi *et al.* (1989), and Shirlaw and Ashford (1962). In contrast to Alhendi *et al.* (1993), De la Fuente and Suarez, (1985a) and Nasreen *et al.* (2007) who claimed that abscesses were located within lymph

nodes. However, micro-abscessation of lymph nodes, which is considered to provide evidence for the hypothesis that abscesses develop within lymph nodes, was not described.

Whether the abscesses detected at shearing time in some ewes were caused by *S. aureus subsp. anaerobius* and whether they were the source of transmission to the young sheep group could not be determined, since the abscesses had healed when the flock was examined. Abscess disease affects mainly young animals up to about 9 months of age as agreed with De la Fuente *et al.* (1997d) and Møller *et al.* (2000) who stated that Subcutaneous abscesses were observed in imported 4- to 5-month-old lambs of the Lacaune breed 10 days after arrival in Denmark. However, in an epidemiological study Tejedor *et al.* (1991) found that between 0.5 and 2.5% of 1-2 year old sheep were affected by abscess disease, and they suggested that these animals might act as reservoirs and maintain the bacteria between outbreaks. Our association of the outbreak with shearing was mainly based on the incubation period required for abscesses to be clinically detectable. Ruiz Santa Quiteria *et al.* (1994) could reproduce the disease in lambs and kids by rubbing the superficial skin incisions with a swab soaked in an overnight culture of the bacteria. According to these authors, the first signs observed 5-7 days after infection, may go unnoticed under field conditions, and the disease was usually first identified when abscesses are clinically detectable about 3 weeks post-infection. Thus, in the present outbreak, the animals most probably became infected about shearing time and, probably, via shearing cut wounds. The location of abscesses in the natural cases of the disease, as well as the results of experimental infections (Bajmo'cy *et al.*, 1984; Ruiz Santa Quiteria *et al.*, 1994), suggested that the primary portal of entry for *S. aureus subsp. anaerobius* is skin abrasions or wounds.

The results of this study revealed that the isolates of *S. aureus subsp. anaerobius* were resistant to penicillin and erythromycin in contrast to the results of (De la Fuente *et al.*, 1985c) and that might be explained due to presence of many isolates of *S. aureus subsp. anaerobius*. Although the results of sensitivity test revealed that the isolates were classified as resistant to penicillin and sensitive to streptomycin, the diseased sheep responded well to the surgical interference and antibiotic treatment (pen-strep® 20/20 Inj. susp. antibiotics (Procaine penicillin G 200,000 Iu. + Dihydro streptomycin Sulphate 200mg) and that might be explained due to the synergistic action of penicillin and streptomycin against staphylococcal infection

(I M V- Hoge Mauw 900 – B – 2370 Arendonk Belgium). Animals in the studied outbreak were kept under cover, in close contact, for about 2h after shearing. This could have improved the opportunity for *S. aureus subsp. anaerobius* to spread to skin cuts.

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