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***Staphylococcus aureus* Isolated from a Horse in a Sudden Death Condition in Kassala State, Eastern Sudan**

¹M.M. Omer, ²Sarah Abusalab, ¹M.M. Gumaa, ¹S.A. Mulla,
²H.M. Osman, ²Y.A. Sabiel and ¹A.M. Ahmed

¹Kassala Veterinary Research Laboratory (KVRL),
Animal Resources Research Corporation, P.O. Box 237, Kassala, Sudan

²Central Veterinary Research Laboratories (CVRL),
Animal Resources Research Corporation, P.O. Box 8067 (Alamarat), Khartoum, Sudan

Abstract: In this study, *Staphylococcus aureus* was isolated from a draught horse presented to Kassala Veterinary Research Laboratory (KVRL) for investigation and diagnosis. The affected animal was previously treated with Gentamycin and Tetracycline before submitted to this laboratory, but the animal was not cure. Thereafter, the animal was attended to the laboratory; it was suffering from inguinal abscess and some clinical signs. Clinical and laboratory examinations were carried out however, the animal was suddenly fallen and died immediately. Then post-mortem, bacteriological and histopathological examinations were done. *S. aureus* was isolated as pure culture from the lungs and peritoneum of the necropsied animal. This isolate showed highly sensitivity to Erythromycin and Clindamycin and it was resistant to Penicillin, Cloxacillin, Cefotaxime, Co-Trimoxazole, Cephalexin and Tetracycline. It was concluded from this study, the shock resulted from *S. aureus* was the causative agent of the death in this case.

Key words: *Staphylococcus aureus*, horse, sudden death, Sudan

INTRODUCTION

Staphylococcus was firstly described in pus from pyogenic infections of humans by Alexander Ogston in 1880. *S. aureus* is Gram-positive cocci. The cocci are mainly arranged in grape-like clusters but some, especially when examined in pathological specimens, may occur as single cells or pair of cells. The principal pathogen of the genus is *S. aureus* which causes a wide range of major and minor infections in man and animals. It has been also associated with pyogenic and toxin-mediated infections in many animal and human conditions. It is characterized by its ability to clot blood plasma by action of the enzyme Coagulase (Greenwood *et al.*, 2002). It is a commensal of the skin and mucous membranes, especially of the upper respiratory and digestive tracts of humans and other animals. Human beings may become infected with *S. aureus* and possibly by other *Staphylococci* of animal origin (Carter, 1986). The present study was carried out to provide the actual information of *Staphylococcus aureus* isolated from this case.

Case history: In July 2007, a draught horse about 10 years old was submitted to Kassala Veterinary Research Laboratory (KVRL) for investigation and diagnosis. The

animal was suffering from inguinal abscess in the left hind quarter. Then after a course of I/M Gentamycin broad spectrum, the animal showed slight improvement. Later on an antiseptic (Potassium Permanganate) and local Oxytetracycline Hydrochloride powder 100% were also used. All these drugs were given to the sick animal before it has been attended to the laboratory, but the animal was not cured. After that the animal was presented to KVRL in a poor body condition and with some clinical signs. However, several diagnostic tests were negative for internal parasites, blood parasites and brucellosis. The animal was also free from external parasites. The affected animal was suddenly fallen and has been recumbent. Many trials for resuscitation were done, but the animal died immediately.

MATERIALS AND METHODS

Time of the study: This study was carried out in July 2007.

Area of the study: KVRL where the case was reported is about 7 km from Kassala town.

Clinical manifestations: The sick animal was examined for clinical signs, location and progress of lesions.

Post-mortem examinations: The necropsied animal was examined systemically and then any visual pathological changes in the internal organs were noted.

Bacteriological samples: Specimens from the lungs, peritoneum and liver were collected aseptically from autopsied animal for bacteriological examination. Impression smears from the specimens were stained with Gram's Method. All samples were cultured onto Blood and MacConkey Agar media and incubated aerobically at 37°C for 24 h, while the liver specimens were also incubated anaerobically for 48 h. After incubation cultures were examined with the naked eye for growth, colony type and any changes in the media. Identification of the isolates was biochemically done according to methods described by Barrow and Feltham (1993).

Antibiotic sensitivity test: Antibiotic susceptibility test was determined by disk diffusion method according to National Committee for Clinical Laboratory (NCCLS, 1993). The multidisk (Axiom, MD018) was used in the study included the following antibiotics: Penicillin G (P₁₀), Co-Trimoxazole (BA₂₅), Cephalexin (PR₃₀), Tetracycline (TE₃₀), Cefotaxime (CF₃₀), Ciprofloxacin (CP₅), Pefloxacin (PF₅), Ofloxacin (OF₅), Cloxacillin (CX₅), Erythromycin (E₁₅), Clindamycin (CD₂) and Gentamycin (GM₁₀). Concentration of all these antibiotics was in micrograms while Penicillin G in units. The inhibition zone of the growth was measured in millimeters (mm).

Histopathological samples: Specimens from lungs, peritoneum, liver and spleen were fixed in 10% formalin and were sent to the Central Veterinary Research Laboratory (CVRL) for histopathological examination.

RESULTS

Clinical findings: The clinical picture was manifested by dullness, complete anorexia, dry muzzle and emaciation. Other signs involved pain and discomfort.

The inguinal abscess was the obvious lesion which reflected by the abduction of the hind quarters of the affected animal (Fig. 1).

Post-mortem findings: At necropsy, the most prominent lesions were seen in the liver, spleen, lungs and peritoneum. The organs were congested and showed petechial hemorrhages. The liver was enlarged, friable, pale color in some areas, contained necrotic foci and mottled with ecchymotic hemorrhages (Fig 2). The lesions seen in the lungs were ecchymotic hemorrhages, emphysema and unilateral hepatization (Fig. 3).



Fig 1: Abduction of the hind quarters of the sick animal due to the inguinal abscess



Fig 2: The liver of the animal was mottled with ecchymotic hemorrhage, pale in color and contains necrotic foci



Fig 3: The lung of the affected animal showed hepatization of the apical lobe, ecchymotic hemorrhage and emphysema

Adhesions and slight enlargement were noticed in the spleen. There was severe peritonitis, exudates, yellowish color and prominent blood vessels were observed in the peritoneum.

Bacteriological examination: The impression smears from lungs and peritoneum showed Gram-positive cocci, while microorganisms were not detected in liver impression smears. Cultures from lungs and peritoneum onto blood agar plates revealed pure colonies, but no growth was obtained from liver either aerobically or anaerobically. Gram-stained smears from these colonies yielded Gram-positive cocci. All cultures onto MacConkey Agar plates were negative for bacterial growth. According to Barrow and Feltham (1993), the isolates were identified as *Staphylococcus aureus*.

Antibiotic sensitivity test: The diameter of inhibition zone of each antibiotic to the isolate as follows: Erythromycin (E_{15}) = 25, Clindamycin (CD_{20}) = 24, Ciprofloxacin (CP_5) = 23, Ofloxacin (OF_5) = 21, Gentamycin (GM_{10}) = 20 and Pefloxacin (PF_5) = 20, Cephalexin (PR_{30}) = 11 and Tetracycline (TE_{30}) = 10 mm. However, no inhibitory zones were observed in Penicillin G (P_{10}), Cloxacillin (CX_5) and Cefotaxime (CF_{30}) and Co-Trimoxazole (BA_{25}).

The isolate was sensitive to Erythromycin, Clindamycin, Ciprofloxacin, Ofloxacin, Gentamycin and Pefloxacin, but it was also resistant to Penicillin G, Cloxacillin, Cefotaxime, Co-Trimoxazole, Tetracycline and Cephalexin.

Histopathological findings: The most striking histopathological changes were seen in the liver, lungs and spleen. The liver was inflamed, necrotic and dilated of the sinusoids, while hemosiderin was observed in the spleen. Emphysema and interstitial pneumonia with infiltrations of leucocytes and macrophages were seen in the lungs.

DISCUSSION

Isolation of *S. aureus* from humans and animals in the Sudan was reported by many researchers (Wisal *et al.*, 2005). Most of these researches showed that isolation of the organism from horses in the Sudan seems to be lacking however, the organism has been isolated from these animals in many countries (Weese, 2004; Vengust *et al.*, 2006). The present condition appears to be the first reported case of *S. aureus* associated with a sudden death of a horse in the Sudan.

S. aureus causes pyoderma in horses, suppurative infections and septicemia in all animals. Moreover, the

organism causes a wide variety of diseases; the major effects of this organism include enterotoxins that cause both of food poisoning and toxic shock syndrome (Orwin *et al.*, 2003). In the present study many reasons might be attributed to incriminate *S. aureus* in causation of the death in this case. Inguinal abscess was the main problem in this case therefore; *S. aureus* was the principal pathogen which causes abscesses in humans and different animal species (Greenwood *et al.*, 2002). In this study *S. aureus* was isolated from the lungs and peritoneum of the affected animal, the isolate was identified as *S. aureus*. Approximately 50% of *S. aureus* strains produce one or more enterotoxins and strains of it produce coagulase (Carter and Darla, 2003). In addition, isolation of the organism from these organs and the absence of other bacteria, this emphasized that the organism act as a cause of this case. Since the organism was isolated from different organs of the sick animal metastasis of the organism from the inguinal abscess might be expected. The death of the affected animal was happened suddenly in a sporadic case associated with shock condition; this shock might be due to toxins produced by *S. aureus* as noted previously by Orwin *et al.* (2003). The inflammatory changes and necrosis were observed in different organs, this in agreement with (Wang *et al.*, 2004) who reported typical lesions caused by *S. aureus*. Environmental factors such as heavy rains in this year might play a role in such case therefore, *S. aureus* was able to survive and multiply in the moist condition (Greenwood *et al.*, 2002). In addition the sick animal was not only emaciated but also used as a draught animal; this fact reinforces the belief that *Staphylococci* cause the disease in predisposed animal (Greenwood *et al.*, 2002). Factors like bad management system and animal husbandry method possibly have a significant role in this case. From all these reasons *S. aureus* seems to be the pathogenic organism in this condition.

The antibiotic sensitivity test showed that *S. aureus* was highly sensitive to Erythromycin and Clindamycin and resistant to a large number of antibiotics such as Penicillin, Cloxacillin, Cefotaxime and the other antibiotics which have been mentioned previously. The resistance of the isolate to Penicillin is attributable to penicillinase (beta-lactamase) an enzyme that hydrolyses the beta-lactam ring of Penicillin (Henry, 2001). However, the isolate was sensitive to Cephalosporin which contains the beta-lactam ring; the sensitivity of the isolate to this antibiotic is a matter of discussion. Levy (1994) mentioned that an antimicrobial use and misuse have contributed to the emergence and spread of antimicrobial resistant microorganisms.

Due to the variation in sensitivity of the organisms to the drugs used, a combination of antibiotics is recommended for the treatment in this case. Further studies are needed to determine the staphylococcal infections in different animal species in Kassala state.

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