

Aerobic Bacteria Associated with External Wounds of Donkeys in Khartoum State, Sudan

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المخلص

فُحصت في هذه الدراسة جروح خارجية لـ 808 حمار بولاية الخرطوم لعزل البكتيريا التي تلوث الجروح. شمل التقصي التعرف علي البكتيريا المسببة للتلوث وإجراء اختبار حساسيتها لست من المضادات البكتيرية. شملت المعلومات التي جمعت عن الحمير العمر والجنس ونوع العمل والموسم وعدد ساعات العمل وموقع الإصابة في جسم الحيوان ونوع العلاج. وُجدت الجروح في 249 حيوان (30.8%) من الحيوانات المستقصاة. عُزل خمس وعشرون نوعاً تنتمي إلي 18 جنساً من البكتيريا، هي: المكورة الحمراء (31.68%)، المكورة العنقودية (31.06%)، المكورة الدقيقة (12.11%)، الوندية (5.59%)، الشعية (5.59%)، العصوية (4.96%)، المكورة السبحية (2.8%)، الباسترية (1.86%)، البيركولديرية (1.24%)، النيسرية (1.24%)، الراكدة (0.93%)، المغزلية (0.93%)، الليسترية (0.62%)، العصية الشعية (0.62%)، البرسينية (0.62%)، المشعشة (0.31%) و البروسية (0.62%) و الصُفيرية (0.31%) و المانهيمية (0.31%). أُختبرت حساسية خمسة عشر نوعاً من البكتيريا لست من المضادات البكتيرية. كانت البروسية المجهزة مقاومة لجميع المضادات المختبرة بينما وُجدت المكورة الخيلية حساسة للأميسلين وقليلة الحساسية للنتراسيكلين. أوضحت عمليات التقصي أن 45.9% من الحيوانات الجريحة تقل أعمارها عن 8 سنوات كما وجد أن 35.54% من الإصابات قد حدثت في فصل الصيف. توزعت معظم الجروح علي الظهر (71.55%) ثم في البطن (14.22%) و الأرجل (11.5%). توجد علاقة مباشرة بين موقع الجرح في جسم الحيوان ونوع عمل الحيوان. تقاسم كل من العلاج بالعقاقير البيطرية والعلاج التقليدي علاج معظم الحيوانات المصابة. كذلك بينت الدراسة بأن 59.44% من الحيوانات المصابة عانت من أمراض جلدية أولية منها 35.09% أصيبت بخراريج تحت الجلد بينما 20.48% من الحمير عانت من أمراض جلدية ثانوية مثل ناسور الحارك نسبة 14.6%.

Summary

In the present study, 808 donkeys in Khartoum State were investigated for wounds bacterial infections. The investigation included isolation and identification of the contaminating bacteria, and determination of their sensitivity to different antibacterial drugs. The information collected included, age, sex, type of work, season, duration of work, site of wound and type of treatment. Wounds were detected in 249 (30.8%) of the investigated animals. Twenty-five bacterial species were isolated. The isolates were identified as *Rhodococcus equi* (31.68%), *Staphylococcus* spp. (31.06%), *Micrococcus* spp. (12.11%), *Corynebacterium* sp. (5.59%), *Actinomyces* spp. (5.59%), *Bacillus* sp (4.96%), *Streptococcus* spp. (2.8%), *Pasteurella* sp. (1.86%), *Burkholderia* sp. (1.24%), *Neisseria* sp. (1.24%), *Acinetobacter* sp. (0.93%), *Fusobacterium* sp. (0.93%), *Listeria* sp. (0.62%), *Brucella* sp. (0.62%), *Actinobacills* sp. (0.62%), *Yersinia* sp. (0.62%), *Flavobacterium* sp. (0.31%) and *Mannheimia* sp. (0.31%). Fifteen bacterial species were tested for their sensitivity to six antibacterial drugs. *Brucella abortus* was resistant to the all tested antibiotics. *Rhodococcus equi* was resistant to ampicillin and moderately sensitive to tetracycline. The collected data revealed that 45.9% of the wounded donkeys were less than 8-year-old and 35.54% of them were wounded during summer. Most of the wounds (71.55%) were present on the back of the animal, 14.22% at abdominal region and 11.5% on the legs. There is direct relation between the site of wound and type of work, and most of the wounded animals were either treated with traditional methods or drugs. The study revealed that 59.44% of infected animals suffered from primary skin diseases; 35.09% of them suffered from subcutaneous abscesses whereas 20.48% of infected animals suffered from secondary skin diseases of which 14.60% had fistulous withers.

Introduction

The importance of donkeys is increasing in the Sudan as well as in many African countries (Hatenorth and Diller, 1986). The owners suffer when their donkeys fall ill or die, so the health of donkeys is important. In all countries, donkeys suffer from being used for work too young, or with damaged legs (Soulsby, 2000). The skin diseases in large animals are primary and secondary. A primary disease is any disease of skin characterized by inflammation due to bacterial infection. Secondary diseases include glanders caused by *Burkholderia mallei* (formerly *Pseudomonas mallei*), a zoonotic infection in foals caused by *Rhodococcus equi*, Strangles caused by *Streptococcus equi*, Fistulous withers caused by *Brucella abortus*, *Actinomyces viscosus*, *Streptococcus zooepidemicus* and *Staphylococcus aureus*, which were isolated from horses. In addition, septicemic pasteurellosis, caused by *Pasteurella multocida* was reported in horse and donkey in India, Listeriosis caused by *Listeria monocytogenes* causes septicemic disease in horses, *Streptococcus equisimilis* is commonly isolated from skin and vagina of horse (Blood et al., 2000).

The objectives of this study were to estimate the incidents of external wounds in donkey in Khartoum State, to identify and classify the bacteria as causative agents of primary and secondary skin diseases and to determine the sensitivity of the isolates to different antibacterial drugs.

Materials and Methods

Experimental design

Eight hundred and eight donkeys were investigated for the presence of external wounds during May 2003 to October 2004. The field work was carried out in Khartoum State (Khartoum, Khartoum North and Omdurman). Two hundred and forty-nine samples were collected from 249 donkeys suffering from external wounds. Sterile swabs were used for collection of samples from the inside of the wounds, placed in sterile tubes, preserved on ice and transported to the laboratory within 4-6 hrs.

Samples were streaked onto fresh Blood Agar plate medium and incubated aerobically at 37 °C for 1-3 days. Isolated bacteria were purified by repeated subculture on Blood Agar plates

and incubated as previously described. Identification of isolates was carried out according to Barrow and Feltham (2003).

Data were collected using a questionnaire, that included serial number, locations (Khartoum, Omdurman or Khartoum North), sex, age, season of collection (Summer, Winter or Rainy), veterinary service (available or not), type of work (public transport, carrying water, distribution of milk or both public transport and water), duration of work (less or more than seven hours), site of wound (head, abdomen, back or legs) and treatment (drugs, traditional medicines or no treatment). Data were analyzed by SPSS

Result

From 808 investigated donkeys, 249 were found wounded (30.8%). With regards to location of the surveyed donkeys, 47.65% were from Omdurman area; whereas the highest percentage of wounded animals was in Khartoum North (Table 1). Regarding the age, 45.9% of wounded animals were less than 8-year-old, 28.3% were between 8-16 years of age and 17.9% were above 16 years of age. It was found that 71.55% of the wounds were on the back of the animal. Seasonally, the percentages of affected animals were 35.54%, 23.91% and 27.43% in summer, rainy and winter seasons respectively. Veterinary service was available for only 34.9% of investigated animals of which 23.4% were wounded. For the type of work, the survey revealed that, 332 were used for carrying water, of which 15.66% were wounded, whereas, 230 of the investigated animals were used for public transport, of which 51.73% were wounded, beside 120 of donkeys were used for milk distribution, of which 4.16% were wounded. It was found that, 715 donkeys (88.49%) were forced to work for more than 7 hrs/day. A percentage of 44.9 of wounded animals were treated by traditional medicines.

Two hundred and forty-nine samples from wounded animals were investigated bacteriologically. Bacteria were isolated from 245 samples and 322 isolates were obtained. The isolates represented 18 different genera, with *R. equi* as the most frequently isolated

bacterium (Table 2). Forty-five isolates representing 15 bacterial species (12 genera) were subjected to sensitivity test (Table 3). Most of them were found sensitive to gentamicin (93.3%), co.trimoazole (80%) and chlormphenicol (88.8%), whereas 86.66% of isolates were found resistant to ampicillin and

showed variable degrees of susceptibility to tetracycline. *Brucella abortus* was resistant to all antimicrobials tested. *R. equi* was found sensitive to co.trimoazole, gentamicin and ofloxacin, moderately sensitive to tetracycline and resistant to ampicillin (Table 3).

Table 1: Numbers of investigated donkeys in Khartoum State, Sudan

Location	Investigated donkeys	Wounded donkeys (%)
Khartoum North	133	72 (54.13)
Omdurman	385	56 (14.54)
Khartoum	280	121 (45.21)
Total	808	249 (30.8)

Table 2: Bacterial species isolated from donkeys' wounds

Bacterial species	Total number of isolates	Frequency of isolates (%)
<i>Rhodococcus equi</i>	102	31.68%
<i>Staphylococcus equorum</i>	50	15.53%
<i>Staphylococcus aureus</i>	33	10.25%
<i>Micrococcus varians</i>	22	6.83%
<i>Staphylococcus hyicus</i>	17	5.28%
<i>Micrococcus roseus</i>	17	5.28%
<i>Bacillus mycoides</i>	16	4.97%
<i>Actinomyces pyogenes</i>	9	2.80%
<i>Corynebacterium pseudotuberculosis</i>	9	2.80%
<i>Streptococcus equi</i>	5	1.55%
<i>Pasteurella multocida</i>	5	1.55%
<i>Actinomyces bovis</i>	5	1.55%
<i>Actinomyces viscosus</i>	4	1.24%
<i>Burkholderia mallei</i>	4	1.24%
<i>Neisseria lactamica</i>	4	1.24%
<i>Streptococcus zooepidemicus</i>	3	0.93%
<i>Acinetobacter anitratus</i>	3	0.93%
<i>Fusobacterium nerophorum</i>	3	0.93%
<i>Brucella abortus</i>	2	0.62%
<i>Actinobacillus equuli</i>	2	0.62%
<i>Yersinia pseudotuberculosis</i>	2	0.62%
<i>Listeria monocytogenes</i>	2	0.62%
<i>Flavobacterium indologenes</i>	1	0.31%
<i>Streptococcus equisimillis</i>	1	0.31%
<i>Mannheimia haemolytica</i>	1	0.31%

This study revealed that 59.44% of infected donkeys had suffered from primary wound infection. Most of the animals of this category were affected by subcutaneous abscesses (35.09%). Secondary wound infection accounted for 20.48% of infected animals. Most of the affected animals suffered from fistulous withers. Non-pathogenic

bacteria (*Micrococcus* spp. *Acinetobacter anitratus*, *Flavobacterium* sp. and *Bacillus mycoides*) were isolated from 18.32% of the cases in this study. Some isolated bacteria (*Mannheimia haemolytica* and *Neisseria lactamica*) were isolated from 1.55% of the infected animals (Table 2).

Table 3: Sensitivity of certain bacterial species isolated from donkeys' wounds to six antibacterial drugs

Organism	Amikacin 20s mcg	Tetracycline 30 mcg	Ampicillin 20 mcg	Co. Trimoxazole 25 mcg	Gentamicin 10 mcg	Chloronphenicol 30 mcg
<i>Rhodococcus equi</i>	-	M	R	S	S	-
<i>Staphylococcus equorum</i>	-	M	R	S	S	-
<i>Saphylococcus aureus</i>	-	S	R	S	S	S
<i>Micrococcus varians</i>	-	R	R	S	S	-
<i>Corynebacterium pseudotuberculosis</i>	-	M	R	S	S	-
<i>Streptococcus equi</i>	S	R	R	S	S	S
<i>Pasteurella multocida</i>	-	S	S	S	S	S
<i>Actinomyces bovis</i>	S	M	R	S	S	S
<i>Burkholderia mallei</i>	S	M	M	S	S	S
<i>Staphylococcus hyicus</i>	-	M	R	S	S	-
<i>Fusobacterium nerophorum</i>	S	S	R	S	S	S
<i>Brucella abortus</i>	R	R	R	R	R	R
<i>Yersinia pseudotuberculosis</i>	R	R	R	R	S	S
<i>Streptococcus equisimillis</i>	-	M	R	S	S	-
<i>Actinobacillus equuli</i>	-	M	R	R	S	S

Key: (R) Resistant, (S) Sensitive, (M) moderately sensitive and (-) not tested

Table 4: Primary and secondary skin diseases of donkeys in Khartoum State

Primary diseases	Percentage	Secondary diseases	Percentage
Subcutaneous abscesses	35.09	Fistulous withers	14.60
Folliculitis	15.53	Strangles	1.55
Lymphangitis	2.79	Glanders	1.24
Equine staphylococcal dermatitis	2.28	Pasteurellosis	1.55
Thrush	0.93	Listeriosis	0.62
Total of primary diseases	59.44	Sleepy foal disease	0.62
		<i>Streptococcus equisimillis</i> infection	0.31
		Total of secondary diseases	20.48

Discussion

Donkeys in the Sudan play an important role in supporting poor families, thus keeping of these animals in good health is of valuable help to those low-income people. Skin diseases suppress the animal and reduce its ability to work, so successful treatment is very important. Donkeys are treated as small equines but they have differences in physiology, behaviour and management from horses. Successful diagnosis and treatment depend on appreciation of these differences and identification of the causative agent (Soulsby, 2000).

Eight-hundred and eight donkeys were investigated for wounds in this study; 30.8% were suffering from different wounds, of which 54.13% were detected in Khartoum North. The infection is very high, as the work duration continues throughout the daylight and the animal is used for carrying both

people and water. Heavy loads and continuous work are considered the important factors which stress the animals and expose them to injury. Although many veterinary services are available, donkeys' owners prefer treatment of their wounded animals with traditional medicines. In Omdurman, 14.54% of the investigated animals were wounded during winter and 35.45% during summer; this finding indicates a relationship between the temperature and the occurrence of wounds. This study reveals that most of the working animals are between 8-16 years of age. This fact reflects the high life span average of donkeys in the studied cities compared to that in Ethiopia in which the average donkey life span is rarely over 12 years (Sevendson, 1994). The high percentage of back wounds in donkeys (71.55%) contrasts with the finding of Reilly (1995) who has found that most wounds are located

in legs of investigated donkeys. It is reported that carrying heavy weight causes wounds on the legs while wounds on the back and abdominal region of the animal are caused by saddles (Soulsby, 2000). Hard saddles with heavy loads were used for the donkeys and are regarded as the main cause of the wounds both on the back and abdominal region of the animal. Head wounds are attributes to bad bridle and insect bites.

Among the investigated donkeys, high percentage of the wounded is found in animals used for carrying water especially in summer. However, the highest percentage of wounded animals is observed among animals which work in public transport. The lowest percentage of wounded animals is reported in donkeys used for milk distribution (4.16%). Some wounded animals were treated by traditional medicines using *Acaccia nilotica* (garad), ash (ramad), faeces of donkeys, mahogany powder, henna and leaves of some kinds of trees and oils. This study reflects that 59.44% of infected donkeys had suffered from primary wound infections. Most of the affected animals in this category suffered from subcutaneous abscesses (35.09%), which are caused by many agents, of which *R. equi* (31.68%) is the most frequently isolated bacterium, followed by *S. equorum*, the primary cause of folliculitis (15.53%) in donkeys. Secondary wound infection accounted for 20.48% of the infected animals. Most of the animals suffered from fistulous withers (14.6%). There are many causative agents of fistulous withers of which *S. aureus* (Thornton *et al.*, 1983) and *B. abortus* (Gaughan *et al.*, 1998) are worth noting. *Brucella* sp. is considered to be the most important pathogen associated with fistulous withers cases (Steward, 1935) and is commonly present with *A. bovis* (Kimball and Frank, 1945; Roderick, 1948). *Streptococcus equi* and *streptococcus equisimilllis* are isolated for the first time. All previously reported bacterial causes of fistulous withers in donkeys are isolated in this study except the *Streptomyces* sp. which was isolated by

Hammd *et al.* (1998). Glanders and strangles were reported in the Sudan (Anon, 2000). The presence of these two diseases was confirmed in this study. Most of the isolates are found resistant to tetracycline which is the most frequently used antibiotic in this country.

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