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STUDIES ON LYMPHOID TISSUE ABSCESSES IN CAMELS (*Camelus dromedarius*) SLAUGHTERED AT NYALA SLAUGHTERHOUSE, SOUTH DARFOUR STATE, SUDAN.

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المستخلص

تمت هذه الدراسة في مسلخ نيالا بولاية جنوب دارفور في الفترة ما بين 2009-2011 حيث تم فحص عدد 786 جملا نحرت للاستهلاك الانساني,واظهرت النتائج اصابة 92 جمل بالخراجات في العقد اللمفية والطحال. جمعت 103 عينة من الخراجات من الجمال المصابة (اكثر من خراج واحد في بعض الحيوانات)ممثلة في 90 من العقدة اللمفية و13 من الطحال.

عزلت البكتيريا من 86(83.5 %) عينة: 75 عقدة لمفية و 11 طحال. تم عزل 119 نوعا من البكتيريا شملت البكتيريا العنقودية، السبحية, المكورات الداخلية، الانتروباكتر، الاركانوباتريم ، الكورانيوباكتريم، الباستريلا، الاكتنيوباسلس والاكتينومياسس. أحتوت 50(58.1%) الخراجات علي البكتيريا الكورانيوباكتريم, العنقودية والاركانوباكتريم.

تم عزل الانواع الاتية من الجمال لاول مرة بالسودان من خراجات العقد اللمفية: الكوراينوباكنريم ألسريبس, الاركانوباكتريم بيوجينس, الراديوكوكس اكوي والاستربتوكوكس أبارس.

أظهرت الدراسة النسيجية وجود حالات بؤرنخرية صغيرة مع وجود ارتشاح للخلايا الالتهابية, واستنزاف للخلايا اللمفية بالاضافة الي وجود تضغم وضمور للشعرات الدموية اللمفية وتكدسات من الشعرات الدموية الرفيعة ممتلئة بكريات الدم الحمراء ومنفصلة ومحاطة بالانسجة الليمفية.

أوضحت الدراسة أن المسبب الرئسي لخراجات العقد اللمفية والطحال في الجمال في ولاية جنوب دارفور هو الكورانيوباكترم, العنقودية الذهبية والاركانوباكتريم.

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Abstract

This study was carried out at Nyala slaughterhouse South Darfur State, Sudan to determine the prevalence of abscesses in lymph nodes and spleen and identify bacterial species involved in .A total of 786 slaughtered camels were inspected from 2009 to 2011. 92 camels exhibited 103 abscesses with prevalence rate of 12% (more than one abscesses in some camels).Specimens from these abscesses; 90 in lymph nodes and 13 in spleens were obtained for bacterial isolation and histopathological examination.. Growth was observed in 86 of the specimens. A total of 119 bacterial species was isolated and identified as *Corynebacterium* spp. (42.9%), *Staphylococcus* spp. (20.2%), *Arcanobacterium* spp. (11.8%), *Streptococcus* spp. (5.1%), *Pseudomonas aeruginosa* (4.2%), *Micrococcus luteus* (2.5%), *Bacillus* spp. (2.5%), *Enterobacter* spp. (2.5%), *Proteus* spp. (2.5%), *Rhodococcus equi* (1.7%), *Citrobacter rodentium* (1.7%), *Enterococcus durans* (0.8%), *Escherichia coli* (0.8%), and *Actinobacillus lignieresii* (0.8%). Small necrotic abscesses were seen in most histopathological sections of lymph nodes with infiltration of inflammatory cells. Other sections showed depletion of lymphocytic cells (white pulp), and hyperplasia and hypotrophy of blood vessels were also seen. Moreover, amass of thin-walled blood vessels filled with blood and separated by courageous stroma and surrounded by fibrous tissues. Our findings indicate that abscesses in lymph nodes were caused by *Corynebacterium pseudotuberculosis*, *Staphylococcus aureus*, *Arcanobacterium pyogenes*, and *Corynebacterium ulcerans*, whereas splenic abscesses were caused by *S. aureus*, *A. pyogenes*, *C. pseudotuberculosis*, *Streptococcus pyogenes* and *Ps. aeruginosa*. Therefore, *Corynebacterium ulcerans*, *Arcanobacterium pyogenes*, *Rhodococcus equi* and *Streptococcus ubaris* were first reporting in The Sudan as camel lymphoid tissue pathogens.

Key words: Bacteriology, Histopathology, Camels, Lymphoid tissue abscesses, Sudan.

Introduction

The world population of camels is about 20 million animals, mainly in arid zones, of which 15 million camels live in Africa (glipha, 2006). Lymphadenitis occurs in different species of animals and human, it is characterized by abscesses formation in one or more lymph nodes, both superficial and visceral (Lindsay and Lloyd, 1991; Aleman *et al*., 1996; Peel *et al*., 1997; De Zoysa *et al*., 2005). In the superficial form, peripheral lymph nodes swell and abscess, whereas in the visceral form, systemic complications can lead to chronic thinning. In camels, the lymphadenitis has been described in several countries (Radwan *et al*., 1989; Moustafa, 1994; Afzal *et al*., 1996; Wernery and Kaaden, 2002; Zidan and Pabst, 2002; Tejedor *et al*., 2004; Braga *et al*., 2006). *Corynebacteria* spp., *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Rhodococcus equi*, *Actinomyces* (*Arcanobacterium*) spp., *Citrobacter* spp., *Proteus* spp., *Escherichia coli*, and *Bacillus* spp. are the organisms most commonly involved in in abscesses of lymph nodes (Hong *et al*., 1995; Afzal *et al*., 1996; Kilic and Kirkan, 2004; Younan *et al*., 2005; Azmi, 2008; *Kinne et al*., 2011). To our knowledge, no studies have been made on abscesses of lymph nodes and spleens of the dromedary camels in Sudan. Therefore, the purpose of this study was to determine the causative agents of these abscesses in camels in South Darfur State and to record the histopathological changes.

Materials and methods

Specimen collection

A total of 786 camels slaughtered in Nyala, South Darfur State during the period from 2009 to 2011 were used in this study. Before slaughter, each camel underwent a general physical examination and immediately after slaughter, a visual thorough palpation of the superficial, visceral lymph nodes and spleens was made to detect any abscess lesions. A total of 103 specimens comprising 90 abscessed lymph nodes (prescapular, bronchial, mediastinal, mesenteric, supramammary and mandibular) and 13 splenic abscesses were collected using sterile forceps and scissors. Each specimen was divided into two portions, one was placed in sterile plastic bags held on ice and transported to the Department of Bacteriology in Nyala Veterinary Research Laboratory for examination within 2 hours of collection and the second was fixed in buffered neutral formalin 10% and transported to the Department of Pathology in Veterinary Research Institute (VRI) in Khartoum to be embedded in paraffin and sectioned at 5µm thickness. These sections were stained

with Haematoxylin and Eosin (H&E) for histopathological examination (Bancroft and Stevens, 1990).

Bacteriological examination

The surfaces of the affected lymph nodes and spleens were scored with a hot spatula. An incision was made with a sterile scalpel blade, revealing abscesses surrounded by fibrous tissue and containing caseated white or yellowish white pus. Pus was taken using a sterile loop and inoculated into blood agar with 7% defibrinated sheep blood and MacConkey agar plates. The plates were incubated both aerobically and anaerobically (Gas-pack anaerobic system, Cat. no. 70304) at 37 ºC for 24 h. If no growth was observed after 24 h, the plates were incubated for additional 48 -72 h. Bacterial colonies were characterized morphologically (e.g., color, size, and edge), before a single colony was smeared for Gram stain. If the culture had a variety of bacterial colonies, subculturing was performed to obtain pure culture (Carter, 1984; Quinn *et al*., 2002). Nutrient agar and broth (Oxoid), brain heart infusion agar (Biolife), and tryptone soya agar (Biolife) were also used in this study. Identification of bacterial isolates to the species level was performed using biochemical tests (Barrow and Feltham, 1993; Balows *et al*., 1999). In addition, analytical profile index kits (HiMedia Laboratories Pvt. Limited) were also used to identify members of the Enterobacteriaceae (KB003, Hi25), Staphylococci (KB004, HiStaph), and Streptococci (KB005: HiStrep) (Barrow and Feltham, 1993).

Results

A total of 103 lymphoid tissue abscesses were collected; 90 (87.4%) were lymph node abscesses (Fig. 1, 2 and 3) and 13 (12.6%) were splenic abscesses (Fig. 3). Of these, 86 abscesses (83.5%) consisting of 75 lymph nodes and 11 splenic abscesses yielded bacterial colonies that were identified to the species level. The remaining 17 specimens (16.5%) consisting of 15 lymph nodes and two spleen abscess specimens gave no bacterial growth. Out of 119 bacterial species identified, 104 isolates were gram-positive bacteria, including: - *Corynebacterium* spp. (42.9%), *Staphylococcus* spp. (20.2%), *Streptococcus* spp. (5.1%), *Bacillus* spp. (2.5%), *Enterococcus* spp. (0.8%), *Arcanobacterium pyogenes* (11.8%), *Micrococcus luteus* (2.5%), and *Rhodococcus equi* (1.7%). Fifteen isolates were gram-negative bacteria, including: - *Pseudomonas* spp. (4.2%), *Enterobacter* spp. (2.5%), *Proteus* spp. (2.5%), *Citrobacter rodentium* (0.8%), *Escherichia coli* (0.8%), and *Actinobacillus lignieresii* (0.8%).

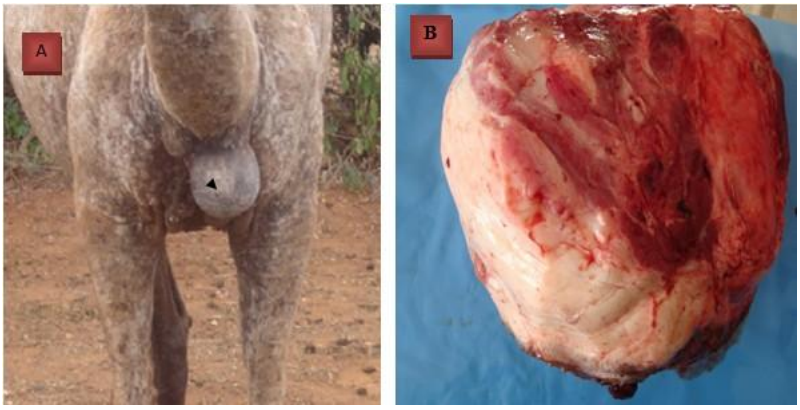

Fig. **(1):** (**A** and **B**) showing enlargement of prescapular lymph nodes abscess of dromedary camel.

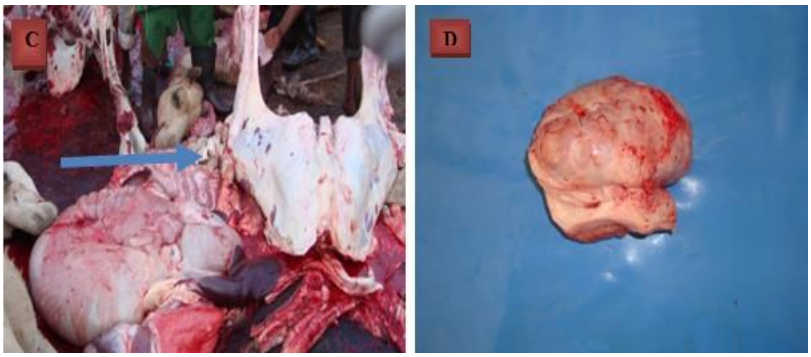

Fig. (2): A camel mesenteric lymph nodes (**C**), A mandibular lymph node (**D**) showing abscesses lesion.

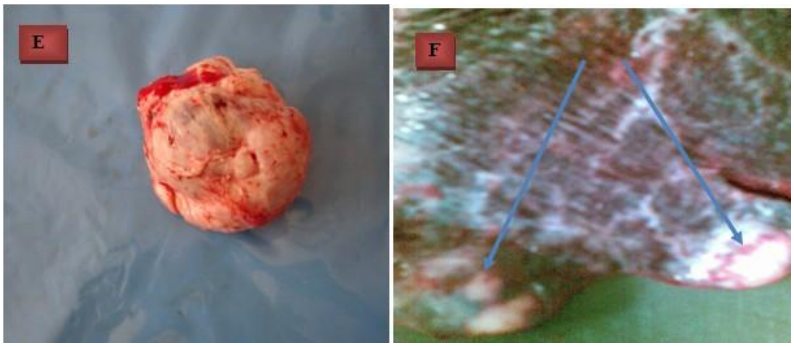

Fig. (**3):** camel bronchial lymph node (**E**) and spleenic (**F**) showing abscess and multi abscesses.

Lymph node abscesses yielded 102 species (85.7%) and splenic abscesses yielded 17 species (14.3%) (Table1). *C. pseudotuberculosis*, *S. aureus*, *S. epidermidis*, *S. intermedius*, *Arcanobacterium pyogenes*, *Strep. pyogenes*, *Ps. aeruginosa*, and *P. vulgaris* were isolated from both lymph nodes and splenic abscesses. *Corynebacterium ulcerans*, *C. diphtheriae*, *S. chromogenes*, *S. kloosii*, *Strep. uberis*, *Strep. equi* subsp. *zooepidemicus*, *Bacillus* cereus, *B. licheniformis*, *Ent. aerogenes*, *Ent. cloacae*, *Micrococcus luteus*, *Enterococcus durans*, *R. equi*, *P. mirabilis*, *C. rodentium* and *A. lignieresii* were isolated from lymph nodes only, whereas *E. coli* was isolated from the spleen only (Table 2). Superficial lymph nodes (prescapular, n=30; mandibular, n=5; supramammary, n=11), and internal lymph nodes (bronchial, n=21; mediastinal, n=14; mesenteric, n=9) yielded 102 bacterial species (Table 3). In some specimens, more than one species were isolated from a single abscess. For example, *C. diphtheriae* was isolated with *S. aureus*, *P.vulgaris* with *C. pseudotuberculosis*, and *E. aerogenes* with *Arcanobacteriu pyogenes*. In the lymph node sections, small necrotic abscesses were seen in the most sections with infiltration of inflammatory cells (Fig. 4). Other sections of lymph node showed depletion of lymphocytic cells (white pulp) due to bacterial infection (Fig. 5). Hyperplasia and hypotrophy of blood vessels and thin-walled blood vessels filled will blood separated by courageous stroma and surrounded by fibrous tissues were seen (Fig. 6).

Table 1 Bacteria isolated from lymph node and splenic abscesses of dromedary camels in South Darfur State.

Bacterial isolates	Lymph nodes, n = 90	Spleen, n = 13	Total (%)
Corynebacterium spp.	49	2	51 (42.9%)
Staphylococcus spp.	18	6	24 (20.2%)
Arcanobacterium spp.	11	3	14 (11.8%)
Streptococcus spp.	4	2	6 (5.1%)
Pseudomonas spp.	3	2	5 (4.2%)
Micrococcus spp.	3	-	3 (2.5%)
Bacillus spp.	3	-	3 (2.5%)
Enterobacter spp.	3	-	3 (2.55)
Proteus spp.	2	1	3 (2.5%)
Rhodococcus equi	2	-	2 (1.7%)
Citrobacter rodentium	2	-	2 (1.7%)
Enterococcus spp.	1	-	1 (0.8%)
Escherichia coli	-	1	1 (0.8%)
Actinobacillus lignieresii	1	-	1 (0.8%)
Total	102 (85.7%)	17 (14.3%)	119 (100%)

Table 2 Bacteria isolated from lymph node and splenic abscesses of dromedary camels in South Darfur State.

Bacterial isolates	Lymph nodes, n = 90	Spleen, n = 13	Total (%)
Corynebacterium pseudotuberculosis	40	2	42 (35.3%)
Corynebacterium ulcerans	8	-	8 (6.7%)
Corynebacterium diphtheriae	1	-	1 (0.8%)
Staphylococcus aureus	12	4	16 (13.4%)
Staphylococcus epidermidis	2	1	3 (2.5%)
Staphylococcus chromogenes	1	-	1 (0.8%)
Staphylococcus intermedius	2	1	3 (2.5%)
Staphylococcus kloosii	1	-	1 (0.8%)
Arcanobacterium pyogenes	11	3	14 (11.8%)
Streptococcus pyogenes	2	2	4 (3.4%)
Streptococcus equi ssp. zooepidemicus	1	-	1 (0.8%)
Streptococcus uberis	1	-	1 (0.8%)
Pseudomonas aeruginosa	3	2	5 (4.2%)
Micrococcus luteus	3	-	3 (2.5%)
Enterococcus durans	1	-	1 (0.8%)
Bacillus cereus	1	-	1 (0.8%)
Bacillus licheniformis	2	-	2 (1.7%)
Rhodococcus equi	2	-	2 (1.7%)
Proteus mirabilis	1	-	1 (0.8%)
Proteus vulgaris	1	1	2 (1.7%)
Enterobacter aerogenes	2	-	2 (1.7%)
Enterobacter cloacae	1	-	1 (0.8%)
Escherichia coli	-	1	1 (0.8%)
Citrobacter rodentium	2	-	2 (1.7%)
Actinobacillus lignieresii	1	-	1 (0.8%)
	102 (85.7%)	17 (14.3%)	119 (100%)

Table 3 Distribution of bacteria isolated from lymph node abscesses of dromedary camels in South Darfur State

Lymph nodes	**A**	**B**	**C**	**D**	**E**	**F**	Total
No. of specimens	30	21	14	9	11	5	90
Bacterial isolates							
Corynebacterium pseudotuberculosis	19	8	7	2	1	3	40
Corynebacterium ulcerans	4	1	1	-	-	2	8
Corynebacterium diphtheriae	-	-	1	-	-	-	1
Staphylococcus aureus	3	5	4	-	-	-	12
Staphylococcus epidermidis	-	1	1	-	-	-	2
Staphylococcus chromogenes	1	-	-	-	-	-	1
Staphylococcus intermedius	1	1	-	-	-	-	2
Staphylococcus kloosii	-	1	-	-	-	-	1
Arcanobacterium pyogenes	2	3	1	3	1	1	11
Streptococcus pyogenes	-	1	-	1	-	-	2
Streptococcus equi spp. zooepidemicus	-	1	-	-	-	-	1
Streptococcus uberis	1	-	-	-	-	-	1
Pseudomonas aeruginosa	-	1	1	1	-	-	3
Micrococcus luteus	2	1	-	-	-	-	3
Enterococcus durans	-	1	-	-	-	-	1
Bacillus cereus	1	-	-	-	-	-	1
Bacillus licheniformis	-	1	-	-	-	1	2
Rhodococcus equi	-	1	1	-	-	-	2
Proteus mirabilis	-	1	-	-	-	-	1
Proteus vulgaris	1	-	-	-	-	-	1
Enterobacter aerogenes	-	1	-	-	-	-	2
Enterobacter cloacae	-	1	-	-	-	-	1
Citrobacter rodentium	-	-	-	1	1	-	2
Actinobacillus lignieresii	-	-	-	-	-	1	1
Total	35	30	17	8	3	9	102

A= Prescapular **B**= Bronchial**C**= Mediastinal **D**= Mesenteric **E**= Supramammary **F**= Mandibular

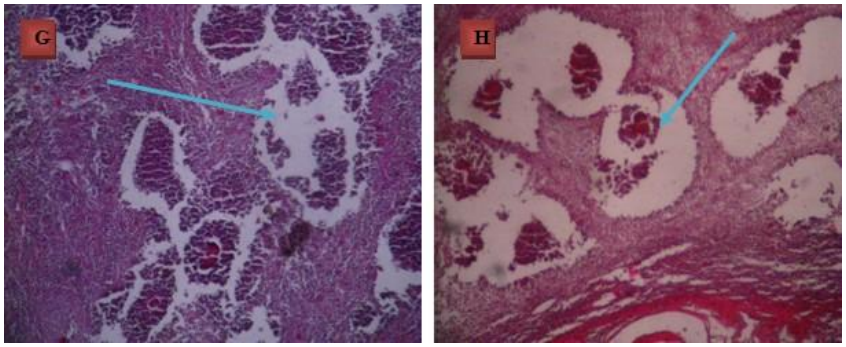

Fig. (4): Camel Lymph node sections (G&H) showing small abscesses area. H&E stain, x100 magnification

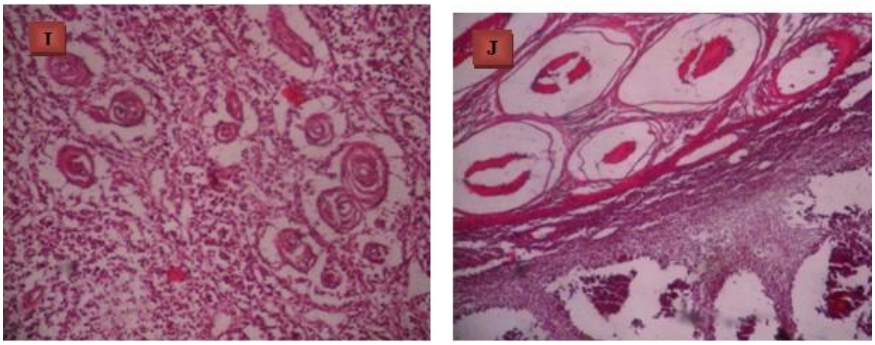

Fig. (5): Camel lymph nodes section showing hyperplasia and hypotrophy of blood vessels (**I**) and amass of this-walled blood vessels filled with blood separated by courageous stroma and surrounded by fibrous tissues (**J**). H&E stain, x100 magnification

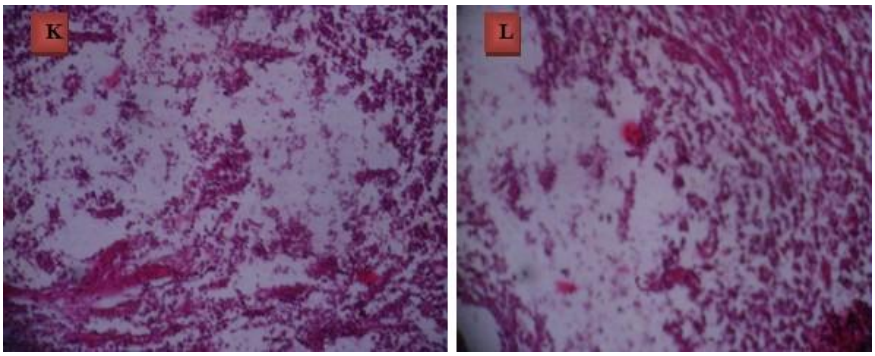

Fig. (6): Camel lymph node sections (K&L) showing depletion of lymphocytes (white pulp). H&E stain, x100 magnification

Discussion

The importance of camels to the economy of the Sudan has led researchers to pay more attention to this animal. This study identified the types of bacteria involved in the lymph nodes and splenic abscesses of camels slaughtered in Nyala. The predominant species obtained were *Corynebacterium* spp. (42.9%), followed by *Staphylococcus* spp. (20.2%) and *A. pyogenes* (11.8%). This finding is in agreement with previous studies that *C. pseudotuberculosis* is commonly isolated from abscesses in the lymph nodes of camels (Radwan *et al*., 1989; Moustafa, 1994; Afzal *et al*., 1996; Braga *et al*., 2006; Azmi, 2008; Tejedor *et al*., 2008). The identification of serotype I and serotype II strongly support the finding of Tejedor *et al*., (2008). These both types affected animals (Biberstein *et al*., 1971; Muckle and Gyles, 1982; Songer *et al*., 1988; Lloyd *et al*., 1990; Mohan *et al*., 2008; Costa *et al*., 1998; Sutherland *et al*., 1996). The isolation of *C. ulcerans* in this study was also consistent with a study conducted by Tejedor *et al*., (2000) who isolated the organism from the dorsal and ventral superficial lymph nodes of the left cervicothoracic region of camels. *A.* (*Actinomyces*) *pyogenes* was isolated from lymph node abscesses in this study; however, Kilic and Kirkan, (2004) isolated *Actinomyces viscosus* from a dromedary camel with large granulomatous nodules on both sides of the postero-ventral side of the mandible. *R. equi* was isolated in this study; this organism was associated diffuse necrotizing lymphadenitis in the bronchial and mediastinal lymph nodes of a 2-year-old male llama (Hong and Donahue, 1995). In addition, the organism was isolated from camel lungs that were diffusely consolidated with large caseous areas (Kinne *et al*., 2011). Radwan *et al*., (1989) isolated *S. aureus*, *R. equi*, *Shigella* spp., and *E. coli* from lymphadenitis in camels. Other bacterial agents isolated in this study, such as *Streptococcus* spp., *Pseudomonas* spp., *M. luteus*, *E. durans*, *Bacillus* ssp., *Proteus* spp., *Enterobacter* spp., *C. rodentium* and *A. lignieresii* have previously been associated with pneumonia and mastitis in the dromedary camel (Barbour *et al*., 1985; Bekele, 1999; Younan *et al*., 2005). Most of the splenic abscesses were caused by *S. aureus*, *A. pyogenes*, *C. pseudotuberculosis* and *S. pyogenes*.

The primary causative agents of abscesses of camels in this region appeared to be *C. pseudotuberculosis*, *S. aureus*, and *A. pyogenes*. Splenic abscesses were by *S. aureus*, *A. pyogenes*, *C. pseudotuberculosis*, *Strep. pyogenes*, *Ps. aeruginosa*, and *E. coli*. Infection with *C. pseudotuberculosis* serotype I and serotype II was due to

husbandry practices, mixed herding, sharing of water and pastures, and migration with other animal species. 17 abscess specimens failed to yield bacterial agents in culture, suggesting that these bacteria were not viable, possibly due to antibiotic use or the abscesses might have been caused by viral, fungal, or parasitic agents. Depletion of lymphoid cells and the presence of RBCs, hyperplasia and hypertrophy of blood vessels in histopathological were similar to findings of Sohair and Eman, (2009).

Conclusion

Our findings indicate that abscesses in lymph nodes were caused by *Corynebacterium pseudotuberculosis*, *Staphylococcus aureus*, *Arcanobacterium pyogenes*, and *Corynebacterium ulcerans*, whereas splenic abscesses were caused by *S. aureus*, *A. pyogenes*, *C. pseudotuberculosis*, *Streptococcus pyogenes* and *Ps. aeruginosa*. Therefore, *Corynebacterium ulcerans*, *Arcanobacterium pyogenes*, *Rhodococcus equi* and *Streptococcus ubaris* were first reporting in The Sudan as camel lymphoid tissue pathogens.

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