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THE MICROFLORA OF TEAT CANALS AND UDDER CISTERNS IN NON-LACTATING DROMEDARIES

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ABSTRACT

The teat canals and mammary gland cisterns of 30 necropsied omniparous non-lactating dromedaries aged between 8 and 20 years were microbiologically investigated. In total 242 teat canals and 242 cisterns were swabbed from which 36 different bacterial species comprising 17 families were isolated. In total 24% teat canals and 22% mammary cisterns harboured bacteria colonies but they grew only in very low numbers of between 1 to 13 colonies per agar plate. It can be stated that our investigation revealed that a high number of dromedary glands are either sterile or harbour only a low number of mainly non-mastitis pathogens.

Key words: Dromedary udder, microflora, non-lactating

Over the last years several scientific papers have been published about camelid mastitis which are summarised by Wernery *et al* (2014) but nothing is mentioned about the mammary glands microflora in non-lactating camels.

The mammary glands of both Old World camels (OWCs) and New World Camels (NWCs) possess four quarters and one teat per quarter. Each teat has at least two streak canals that enter into separate teat and gland cisterns, the latter being a sponge - like structure (Fig 1). About 1 to 2 % of dromedaries possess teats with three streak canals (Fig 2). Mastitis pathogens originate either from the udder (contagious or animal associated pathogens) or from the animal's surroundings (environmental pathogens) such as bedding, manure or soil. It was shown by Wernery *et al* (2008) that mastitis pathogens of the dromedary are the same as cultured from the mammary gland of bovines. These are *Streptococcus agalactiae* (animal associated), *Staphylococcus aureus* (animal associated), Coagulase – negative *Staphylococcus* (CNS) (environmental pathogen), *Streptococcus bovis* (environmental pathogen), *Streptococcus uberis* (environmental pathogen), *Streptococcus dysagalactiae* (environmental pathogen).

The present study intends to evaluate the microflora of teat canals and udder cisterns of non-lactating dromedaries.

Materials and Methods

Animals

The entire udder was removed from 30 dromedaries which were sent for necropsy to the Central Veterinary Research Laboratory in Dubai. The animals which were aged between 8 and 20 years had succumbed to different diseases and were omniparous (at least 2 pregnancies).

Sample collection

The two teat canals and cisterns from each of the four quarters of 30 mammary glands were swabbed separately for bacterial and fungal culture. In total 242 teat canals and 242 cisterns were swabbed as two of the dromedaries possessed 3 streak canals in one of their four quarters (Fig 2). Before a swab was inserted into each streak canal, the tips of the teats were cut off with a sterile disposable scalpel blade after which the streak canals were opened with 2 sterile forceps (Fig 3). A swab was inserted into the canal by rotating the swab many times after which it was put back into Amie's transport media without charcoal. After all swabs were taken, the teat canals were cut open with a sterile scissors exposing the sponge-like cistern for swabbing. Several cavities of each cistern were swabbed with the same swab and transferred into the transport media.

Bacterial and fungal culture

Immediately after collection, the swabs were streaked on bacterial and fungal agars which included:

- Blood agar (Oxoid-CM0055)
- Blood agar (Oxoid-CM0055) for growth of anaerobes under anaerobic incubation using catalysts (Oxoid-Anaerogen AN0025A and Oxoid-anaerobic indicator BR0055B)

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- Nutrient agar (Oxoid-CM003)
- Brilliant-green Phenol-red Lactose Sucrose agar (BPLS-Merck-VM381937213)
- Sabouraud's agar (Merck – VM939415804)

All bacterial agars were incubated for 48 hours at 37°C whereas the fungal agar was incubated at 30°C for 5 days.

The bacterial species isolated were identified with fully automated identification system, Vitek 2 Compact (BioMérieux, USA) and biochemical identification panels – BBL Crystal GP (Becton, Dickinson and Company, USA) and API 20NE (BioMérieux, France).

Results

In total 30 dromedary mammary glands were examined comprising a total number of 120 teats with 242 teat canals (2 dromedaries with one teat having 3 teat canals) as well as 242 cisterns. Of the 242 teat canals microbiologically investigated, 58 (24%) were contaminated with different bacterial species, whereas 187 (76%) were sterile. Only 53 (22%) of the 242 cistern swabs contained bacterial species, whereas 189 (78%) did not reveal any bacterial growth (Table 1). The amount of colonies per blood agar plate was 1 to 13 colonies and no anaerobic bacteria and no fungus was isolated neither from the teats nor from the mammary cisterns.

36 different bacterial species comprising 17 families were cultured from the swabs tested which is summarised in Table 2 along with the number of corresponding quarters of teat canals and cisterns from which they were isolated.

A detailed examination on the distribution of microflora in all the quarters showed, that of the 30 mammary glands examined, 7 were completely sterile with no bacterial growth in any of the swabs collected and 23 were non-sterile harbouring different bacterial species.

In 6, of the 23 non-sterile mammary glands tested, only a single bacterial species was isolated from the swabs collected from different quarters. However, the bacterial species was not equally distributed in the teat canal and cistern quarters. In the first dromedary, *Streptococcus agalactiae* was isolated from the right front teat and cistern swabs, leaving other quarters sterile. *Staphylococcus aureus* with hemolysis was isolated only from the left front and hind cistern swab of second dromedary. Similarly, *Actinomyces* spp was isolated from left hind teat and cistern swab of a third dromedary. *Actinomyces naeslundii* was isolated from left front cistern only of a fourth dromedary and *Staphylococcus simulans* from right front and hind teat swab of a fifth dromedary. In all these dromedaries, no other bacterial species were isolated from the remaining quarter swabs. The above isolated bacterial species are mastitis agents. Their relevance in the study cannot be commented as no clinical history relating to their udder health was available. In a sixth dromedary *Pseudomonas* spp was isolated from all the teat and cistern swabs along with scanty growth of *Staphylococcus* spp. The distribution pattern of bacterial flora in these 6 dromedaries are shown in Table 3.

Multiple bacterial load was observed in the remaining 17 camels. The bacterial species isolated from them were mostly skin and environmental contaminants like, coagulase negative *Staphylococci, Brevibacterium linens, E. coli, Chryseobacterium indologenes, Corynebacterium* spp, *Acinetobacter* spp, *Sphingomonas paucimobilis, Brevundimonas diminuta/ vesicularis, Rhodococcus equi, B. cereus, Streptococcus* spp and *Aerobacilli* (Table 2). From each dromedary almost similar bacterial species were isolated from teat canals and cisterns. However, the bacterial species isolated showed no even pattern of distribution in all the quarters tested (Table 3). No data was available about any predisposing factors for these dromedaries and hence the reason for colonisation or possible route of entry cannot be explained.

Discussion

This study was performed to investigate the bacterial flora of the dromedaries' teat canals and mammary gland cisterns in non-lactating omniparous dromedaries. The mammary gland is protected by a variety of defence mechanisms like innate or specific immunity as well as physiological particularities. Each orifice of the teat canal is sealed by a wax-like plug (Fig 4) of different length which protects the mammary gland from invasion of microorganisms.

In general, teat canals and mammary gland cisterns are free of microorganisms and so is the milk. Under special circumstances they may enter the udder which will initiate a cascade of defence mechanisms to avoid any clinical or subclinical mastitis. In lactating cows, milk somatic cells like neutrophils, macrophages, lymphocytes as well epithelial cells, cytokines including interleukin, interferon and others will help to fight the infection. Furthermore, dromedary milk itself possesses powerful bacterial and virocidal proteins (El-Agamy *et al*, 1992). An

Table 1. Total number of teat canals and cisterns with and without bacterial growth.

Sample	Total	Showing bacterial growth		No growth	
		Number	%	Number	%
Teat canal swabs	242	58	24	184	76
Cistern swabs	242	53	22	189	79

Table 2. Bacterial species and number of corresponding quarters of 242 teat canals and mammary gland cisterns from which they were isolated.

Sl No	Bacterial Species Isolated	Number of teat canal quarters					Number of udder cistern quarters				
		RH	LH	RF	LF	Total	RH	LH	RF	LF	Total
1	*Staphylococcus* species	8	13	7	5	33	8	11	7	5	31
2	*Corynebacterium aquaticum*	1	-	2	5	8	4	5	2	6	17
3	*Acinetobacter lwoffii*	-	3	6	-	9	-	2	2	-	4
4	*Staphylococcus aureus* (with haemolysis)	1	-	1	2	4	-	3	1	2	6
5	*Aerobacilli*	1	1	1	1	4	2	1	2	1	6
6	*Pseudomonas* species	-	1	2	-	3	2	2	2	-	6
7	*Brevibacterium linens*	-	-	2	4	6	1	1	-	-	2
8	*Staphylococcus simulans*	1	1	1	1	4	-	1	2	1	4
9	*Corynebacterium bovis*	2	2	1	-	5	1	1	-	-	2
10	*Moraxella lacunta*	1	-	-	2	3	-	2	1	1	4
11	*Micrococcus* species	-	-	2	-	2	-	2	3	-	5
12	*Corynebacterium confusum*	-	1	1	1	3	1	1	-	-	2
13	*Staphylococcus intermedius* (with haemolysis)	-	2	-	-	2	1	1	-	-	2
14	*Actinomyces* species	1	1	-	-	2	-	2	-	-	2
15	*E.coli*	-	2	-	-	2	2	-	-	-	2
16	*Staphylococcus schleiferi*	-	-	1	-	1	-	-	2	-	2
17	*Chryseobacterium indologenes*	-	2	1	-	3	-	-	-	-	-
18	*Staphylococcus epidermidis*	-	2	-	-	2	-	-	-	1	1
19	*Staphylococcus xylosus*	-	1	-	1	2	-	-	-	-	-
20	*Staphylococcus cohnii* spp *ureolyticus*	-	-	-	2	2	-	-	-	-	-
21	*Streptococcus agalactiae*	-	-	1	-	1	-	-	1	-	1
22	*Corynebacterium amycolatum*	-	-	-	2	2	-	-	-	-	-
23	*Corynebacterium afermentans*	-	-	-	2	2	-	-	-	-	-
24	*Sphingomonas paucimobilis*	-	-	-	-	-	-	1	1	-	2
25	*Brevundimonas diminuta/vesicularis*	-	-	2	-	2	-	-	-	-	-
26	*Rhodococcus equi*	-	-	-	1	1	-	-	-	-	-
27	*Pseudomonas putida*	1	-	-	-	1	-	-	-	-	-
28	*B. cereus*	-	-	1	-	1	-	-	-	-	-
29	*Aerococcus viridians*	1	-	-	-	1	-	-	-	-	-
30	*Staphylococcus warneri*	-	-	-	-	-	-	-	1	-	1
31	*Actinomyces naslundii*	-	-	-	-	-	-	1	-		1
32	*Corynebacterium* species	-	-	-	-	-	1	-	-	-	1
33	*Corynebacterium jeikeium*	-	-	-	-	-	-	1	-	-	1
34	*Alpha –haemolytic Streptococci*	-	-	-	-	-	-	-	1	-	1
35	*Streptococcus infantarius* spp *coli*	-	1	-	-	1	-	-	-	-	-
36	*Acinetobacter baumannii/ calcoaceticus*	-	1	-	-	1	-	-	-	-	-

RH - Right Hind; LH - Left Hind; RF - Right Front; LF - Left Front

Fig 1. A dromedary mammary gland cistern exposing a sponge - like structure.

Fig 2. A dromedary teat with three canals ending in separate cisterns each.

Fig 3. The tip of the teat was cut-off and the teat canals opened with two sterile forceps before swabbing.

Fig 4. Wax-like plug seals the orifice of each dromedary teat canal.

important factor in the aetiology of mastitis in bovine heifers is the fact that a high proportion of teat canals open already several months before calving (Kromker *et al*, 2009).

It is known from high yield cattle dairy farms that mistakes in the hygiene, management and feeding in the early stages of drying cows will consequently favour mastitis in the early lactation period. It is nowadays common practice in dairy cows to apply potent drugs into the teat canals like "Benestermyan, Nafpenzalt or Orbenin extra" shortly before a cow is dried off. The application of dry-off antibiotics has significantly reduced the amount of mastitis cases at the beginning of a new lactation period. Nothing is known about the effect of this approach in camels but should be carefully investigated.

As shown from our investigations, in total 36 different bacterial species comprising 17 families were cultured from the dromedary mammary gland of which only 6 species: *E.coli, Corynebacterium bovis, Staphylococcus aureus, Streptococcus agalactiae, Actinomyces* spp and *coagulase negative Staphylococci* are known mastitis pathogens. In total 24% teat canals and 22% mammary cisterns harboured bacteria colonies but they grew only in very low numbers of between 1 to 13 colonies per agar plate. No anaerobic bacteria and no fungal species were cultivated. The low number of bacteria colonies isolated from dromedary teat canals and cisterns as well as the low percentage of contaminated swabs demonstrates that both, teat canals and cisterns of non-lactating dromedaries are more or less sterile. Although the sampling took place under special sterile conditions, the possibility of contamination of swabs with skin flora cannot be entirely excluded. The results also show that in general similar bacterial species were isolated from all locations but were not equally distributed, with few exceptions. Microbiological investigation of 200 quarter teat tissues in dromedaries by El-Hallawany *et al* (2011) revealed a 36% bacterial recovery rate, although 76.5% of teat tissues showed macroscopic lesions. It should

Table 3. Distribution of bacteria in teat canals and cisterns of 23 dromedaries.

Camel	Teat Canal Swabs								Cistern swabs								Total affected
	RF_1	RF_2	RH_1	RH_2	LF_1	LF_2	LH_1	LH_2	RF_1	RF_2	RH_1	RH_2	LF_1	LF_2	LH_1	LH_2	
1*	●								●								2
2*	●												●		●		3
3	●				●	●											
4							●	●					●				3
5					●				●	●							3
6*													●				1
7							●								●		2
8							●	●							●		3
9	●		●	●	●				●		●	●	●	●			9
10	●	●	●	●	●						●	●			●	●	9
11*							●								●	●	3
12*	●		●														2
13	●	●			●				●		●		●	●			7
14	●		●	●	●		●				●				●		7
15*	●	●					●		●	●	●	●			●	●	9
16			●														1
17			●		●	●	●	●	●				●		●	●	9
18	●	●	●	●	●		●				●				●	●	9
19	●		●	●					●	●	●	●			●		8
20			●				●		●		●	●	●		●		7
21	●								●								2
22		●					●	●									3
23	●				●					●	●						4
Total	18		14		12		14		13		14		9		17		111
	GRAND TOTAL – 58 TEAT CANAL SWABS								GRAND TOTAL – 53 CISTERN SWABS								111

* 6 dromedaries from which a single bacterial species was isolated

be noted that *Rhodococcus equi* which has caused a severe disease in dromedaries (Kinne *et al*, 2011) was isolated from the left front teat of one dromedary.

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