



Epidemiological, Bacteriological and Histopathological Investigations of Mastitis in Camels (*Camelus dromedarius*) in Tumbool Abattoir, Sudan

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ABSTRACT:

A cross-sectional study was conducted in Tumbool abattoir for one year 2009 to 2010 to estimate the prevalence of camel mastitis and its associated potential risk factors. In addition to, isolation and identification of bacterial species that may be associated with mastitis and the histopathological picture of the udder tissues were also assessed. Conventional bacteriological methods, Api kits and the automated system Vitek 2 Compact were conducted to identify the bacterial isolates. Out of eight hundred culled she-camels examined, 660 (82.5%) were adult and 140 (17.5%) were immature. The overall prevalence of the clinically manifested mastitis in adult she-camel was found to be 196 (29.7%). Microscopic examination of 150 sections of udder tissues showed three types of mastitis; acute 33 (22%), chronic 72 (48%) and 45 (30%) were neoplastic masses. Seventy five out of 165 (45.5%) samples showed growth of bacterial colonies after 24 hours incubation at 37°C, with 114 bacterial isolates. The highest frequent isolates were *Staphylococcus* spp. 40 (35.08%), *Streptococcus* spp 29 (25.43%) and *Micrococcus* spp 24 (21.05), and the least isolated bacteria were 1 (0.87%) *Bacillus cereus*, 1 (0.87%) *Mannheimia haemolytica* and 1 (0.87%) *Klebsiella pneumoniae*. Using Pearson Chi-square or Fisher Exact test, risk factors such as age, pregnancy, breed (eco-type), location, tick infestation, anti-suckling device used and bacteria isolated did not show any significant association with the mastitis or the neoplastic masses. The factor found significantly associated with chronic mastitis was udder texture with ($p=0.004$). The likelihood of getting a hard udder in the chronic mastitis (chi-square = 8.423, $p = 0.004$) was 3.61 times more likely than the soft udder. In conclusion, mastitis in camel is prevalent in the country and can be caused by different pathogenic bacteria. Despite the insignificant

association between the examined risk factors and the neoplastic masses revealed by this study. Further research should be tackled in order to determine the role of the neoplastic masses in the disease prevalence for better epidemiological consideration.

Keywords: *mastitis, bacteria, risk factors, camel, epidemiology, histopathology, Sudan*

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INTRODUCTION:

Camels are well adapted to survive in arid zones and are able to withstand thirst and survive on thorny desert shrubs. They are multipurpose animals representing an important source of food (milk and meat), clothing, shelter and transportation for bedwines. Recently camels' milk and urine have proven to have therapeutic effects against certain diseases (Ohaj, 1993, 1998; Salwa, 2004). With the important role of providing milk for human consumption and nourishment to neonates and providing the newborns of many animal species passive protective immunity. However, mammary gland may constitute a source of infection of some pathogens such as *Mycobacterium* and *Brucella*, which are transferred by lymphatic haematogenous resulting in a public health hazard (Schlales and Miller, 2007).

Mammary gland masses are common in bitch and cat, but are rare in other domestic animal species, the mammary masses which is biological behaviour was extensively studied in bitch varied in morphology (Misdrop, 2002). Data regarding udder affections, their etiological agents and prevalence in camelidae are meagre (Abdel Gadir *et al.*, 2006; Kalla *et al.*, 2008). This study was initiated and conducted in response to previous reports which noted that 75%

of female camels slaughtered due to infertility or udder affections (TCRC 2008) to study the bacteriological epidemiology and histopathology and risk factors associated with udder affection of she- camel slaughtered in Tambool abattoir. Different animals from various States are slaughtered in Tumbol abattoir and represent all camel ecotypes of the Sudan.

MATERIALS AND METHODS:

Collection of samples: Out of 800 animals examined, 660 was mature she-camel 196 animal showed udder lesions, 165 samples of them were collected during different seasons for one year from 2009-2010.

The samples were collected aseptically and then transferred to the Department of Bacteriology, Central Veterinary Research Laboratory for bacterial identification and Department of Pathology for histopathological studies.

The isolation and identification of the bacteria were performed using conventional standard methods according to Barrow and Feltham (2004) and Quinn *et al.*, (2003), rapid test using Api 20 strips and the automated system of Vitek 2 Compact.

The histopathological method was done also according to standard method as follow; tissue specimens collected were fixed in 10% formalin solution, processed by standard paraffin embedding

technique, microtomy of the embedded tissue to 5-6 micron thick sections was carried out, then sections were placed onto glass slides, dried and stained with hematoxylin and eosin (H&E).

Data analysis: The univariate association between the histopathological outcomes or the stage of mastitis and the categorical risk factors with two levels was investigated individually by testing its significance using the Pearson Chi-square or Fisher Exact test. Risk factors with more than

Table 1: The bacterial isolates and its frequencies

Bacterial isolates	Frequency (%)
<i>Staphylococcus</i> spp.	40 (35.08)
<i>Streptococcus</i> spp.	29 (25.43)
<i>Micrococcus</i> spp.	24 (21.05)
<i>Enterococcus</i> spp.	5 (4.38)
<i>E. coli</i>	3 (2.63)
<i>Corynebacterium pyogenes</i>	3 (2.63)
<i>Corynebacterium pseudotuberculosis</i>	2 (1.57)
<i>Aerococcus</i> spp.	2 (1.57)
<i>Proteus</i> spp.	2 (1.57)
<i>Bacillus cereus</i>	1 (0.87)
<i>Acinetobacter</i> spp.	1 (0.87)
<i>Mannheimia haemolytica</i>	1 (0.87)
<i>Klebsiella pneumonia</i>	1 (0.87)
Total	114 (100)

Macroscopic examination of 150 udders showed different texture, either soft or hard. The sections of udder showed three types of mastitis viz; acute 33 (22%), chronic 72 (48%) and 45 (30%) neoplastic masses.

Acute type: Grossly, Affected udders examined were markedly swollen with varying in consistency ranging from moderately soft to firm and being painful on palpation; seven udders presented fistulous tracts with oozing pus.

two categorical levels were investigated individually using the univariate logistic regression. All statistical tests were conducted using SPSS version 20 (IBM SPSS) at $\alpha = 0.05$ significance level.

RESULTS:

The prevalence of clinical manifestation of mastitis was found to be 196(29.7%). Seventy five out of 165 (45.5%) samples showed growth of bacterial colonies after 24 hours incubation at 37 °C with a result of 114 bacterial isolates, the results were shown in Table 1.

Histopathologically, oedema and profuse infiltration of cellular exudates, composed of mononuclear cells intermixed with neutrophils in the secretory acini and micro abscesses were also observed. In chronic mastitis: Grossly, affected udders were slightly enlarged and firm in consistency, a few were shrinkage; the most striking histopathological changes in udders were profuse proliferation of fibrous tissue. In certain udders the teat cannals were

folded and occluded by fibrous tissue plug, as mentioned in previous paper (Abeer *et al* 2016).

Neoplastic masses: The most prominent histopathological changes were complete obliteration of normal udder tissue which was replaced by multi-neoplastic masses. The most striking change was complete distortion and destruction of normal

udder architecture and the normal udder tissue was replaced by invasion of tumor tissue. Different five types of tumors were described viz. Intracystic papillary carcinoma, papillary carcinoma, adenocarcinoma, cystic adenomatous hyperplasia and skin papilloma (Figures 1,2,3,4, and 5).

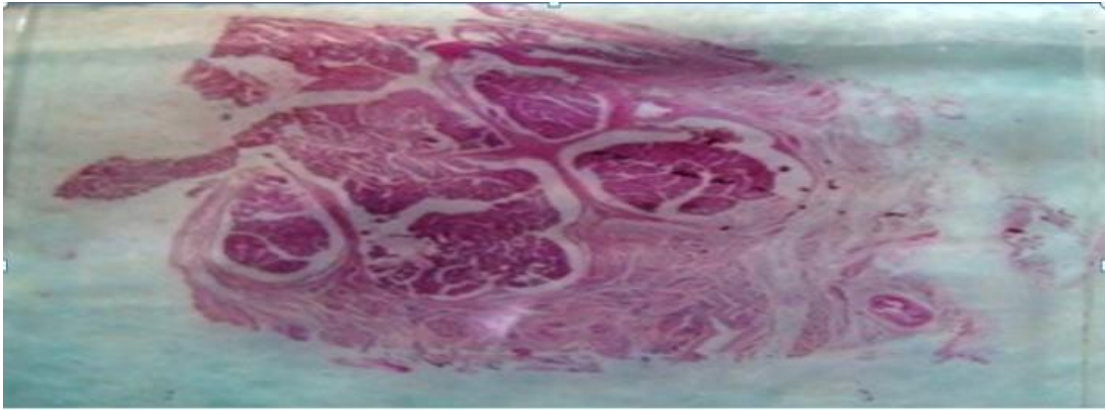


Figure 1: Section of she camel udder affected with intracystic papillary carcinoma

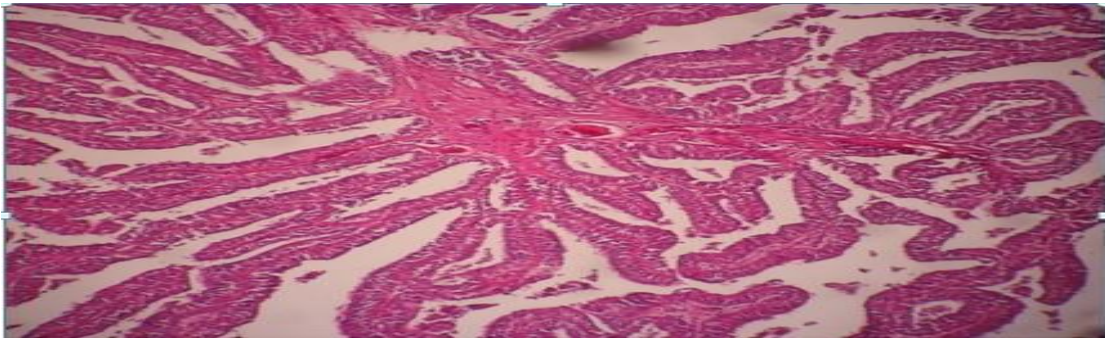


Figure 2: Section of she-camel udder show papillary carcinoma

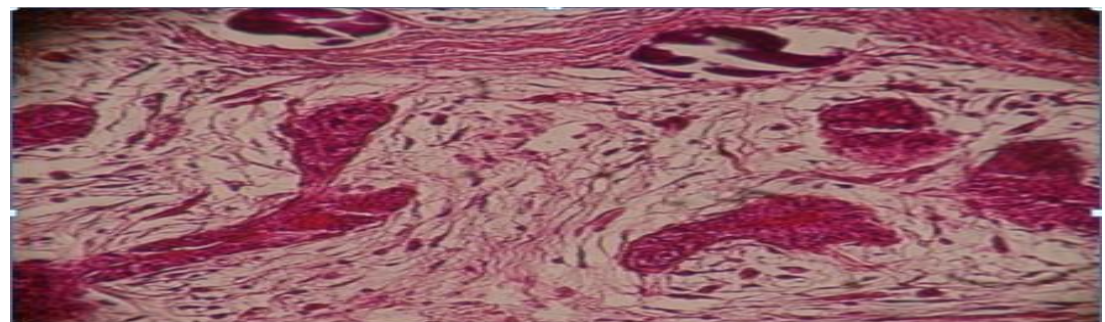


Figure 3: Section of she-camel udder show fibroadenoma

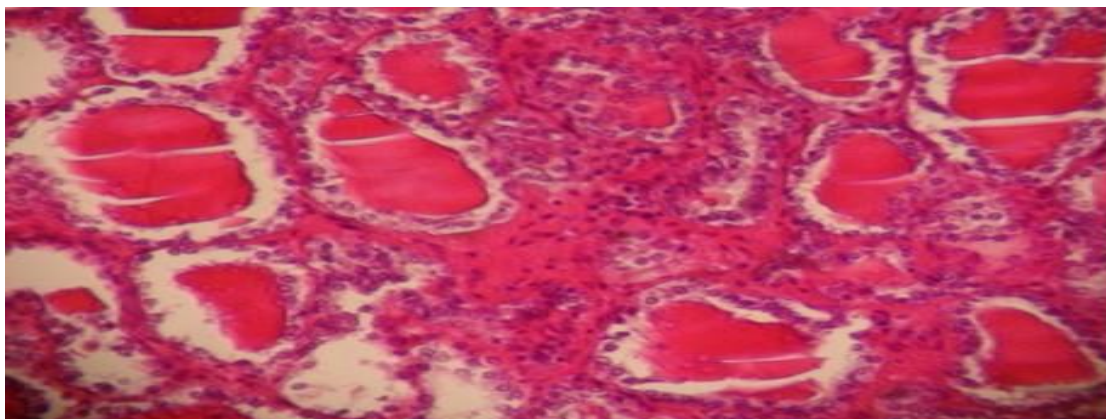


Figure 4: section of she-camel udder show adenocarcinoma

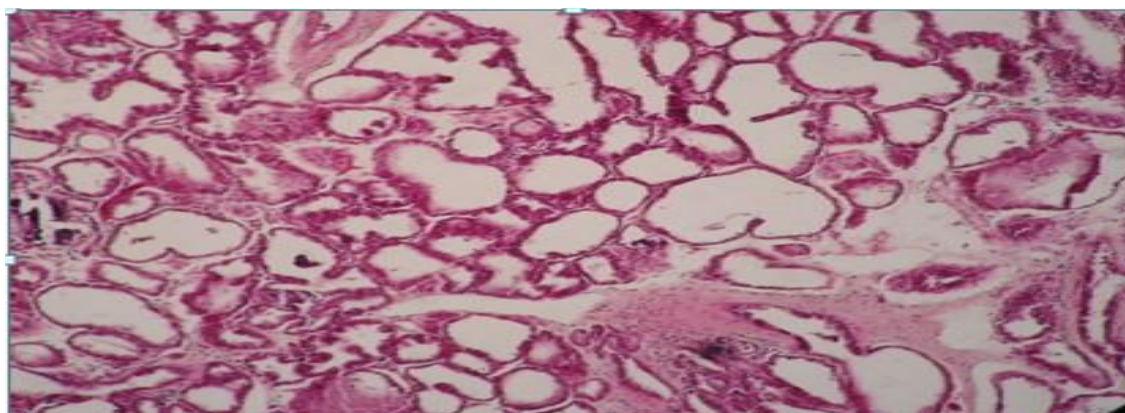


Figure 5: Section of she-camel show adenomatous hyperplasia

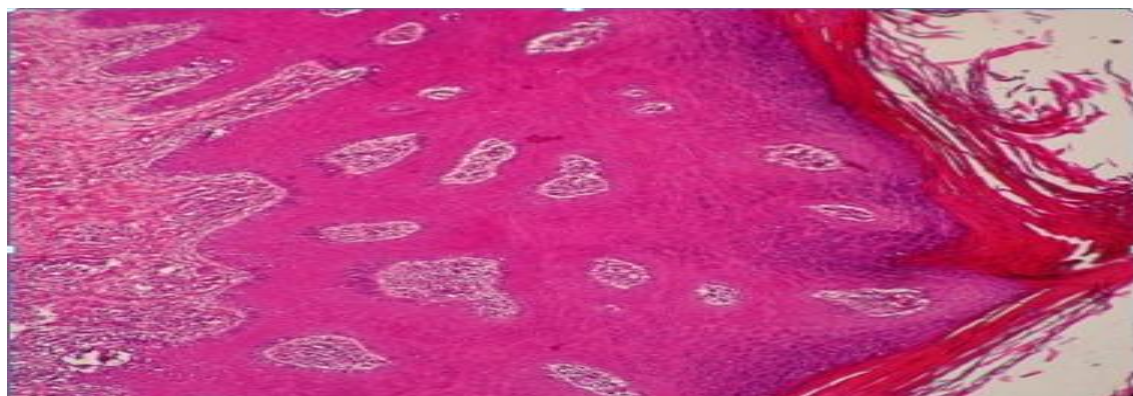


Figure 6: Section of skin of she-camel udder show papilloma

Using Pearson Chi-square or Fisher Exact test, risk factor such as age, pregnancy, breed (eco-type), location, tick infestation, anti-suckling device use and bacteria isolated were not showed significant association with the mastitis

or the neoplastic masses. In the chronic cases of mastitis, the likelihood of getting hard udder (chi-square = 8.423, $p = 0.004$) has 3.61 times more likely than the soft udder, the results in table 2 and 3.

Table 2: Univariate analysis for testing the association of the risk factors with camels histopathological outcomes (mastitis or tumor) in Tumbool abattoir

Risk factors	No.	Histopathological outcome		Chi-square	df	P-value
		Mastitis (%)	Tumor (%)			
Udder status						
Soft	68	48 (70.6)	20 (29.4)	0.001	1	1
Hard	82	58 (70.7)	24 (29.3)			
Ticks infestation						
Heavy	110	77 (70)	33 (30)	0.09	1	0.81
Low	40	29 (72.5)	11 (27.5)			
Pregnancy status						
Yes	31	22 (71)	9 (29)	0.03	1	1
No	119	84 (70.6)	35 (29.4)			
Age						
5-7 years	24	19 (79.2)	5 (20.8)	1.98	2	0.37
>7-10 years	76	55 (72.4)	21 (27.9)			
>10 years	50	32 (64)	18 (36)			
Location						
east	121	86 (71.1)	35 (28.9)	0.05	1	1
west	29	20 (69)	9 (31)			
Anti-sucking use						
yes	124	87 (70.2)	37 (29.8)	0.09	1	0.81
no	26	19 (73.1)	7 (26.9)			
Bacteria isolated						
yes	66	47 (71.2)	19 (28.8)	0.02	1	1
no	84	59 (70.2)	25 (29.8)			

DISCUSSION:

This study was conducted to evaluate the epidemiological, bacteriological and histopathological of culled she-camels due to udder affection. In addition to other factors including ecotype, age, pregnancy, udder status, anti-suckling device and tick infestation. Udder affection induces losses in productive females, including milk, calves, herd's structure and this may lead to economic losses in national herds in Sudan.

In this study 90 (54.5%) of mastitic udder specimens were negative for bacterial growth, this was in line with the results reported by Hawari and Hassawi (2008); bakhiet *et al*; (1992) and

Abdurahman *et al* (1995). This could be due to either that mastitis was chronic and persisted for long period or the udder changed to a fibrotic one, also other causes like viruses, mycoplasma and fungi may contributed. The bacteria isolated in this study were *Staphylococcus* spp. *Streptococcus* spp. *Corynebacterium psudotuberculosis*, *Corynebacterium pyogenes*, *Micrococcus* spp. *Aerococcus* spp. *Bacillus cereus*, *Acinetobacter* spp. *E. coli*, *Mannheimia haemolytica*, *Klebsiella pneumoniae*, *Enterococcus* spp and *Proteus* spp, these were similar to those reported by Mohammed *et al* (2005) and Birhanu *et al* (2008) in

Somalia and Ethiopia. The results were also in line with the finding of Amel (2003) and Sanaa (2005) who found that *Staphylococcus aureus*, *Streptococcus agalactiae*, *micrococcus* spp, *Eschreiea coli*, *Aerococcus*, *Staphylococcus epidermides*, *Corynbacterium* and *Bacillus* spp were the most dominant bacteria isolated from mastatic female

camel. this organism were also isolated from milk of mastitic she-camels in Kurdofan State, Western Sudan (Bakhiet *et al.*, 1992; Obeid *et al.*, 1996 and Ismail 2006), and from Khartoum state and Port Sudan (Suheir, 2004). In one occasion *Brucella* spp was isolated Agab (1993).

Table 3: Univariate analysis for testing the association of the risk factors with the clinical development of udder mastitis in camels in Tumbool abattoir

Risk factors	No.	Clinical mastitis		Chi-square	df	P-value
		Acute (%)	Chronic (%)			
Udder status						
soft	48	22 (45.8)	26 (54.2)	8.84	1	0.003
hard	58	11 (19)	47 (81)			
Ticks infestation						
heavy	77	26 (33.8)	51 (66.2)	0.91	1	0.36
low	29	7 (24.1)	22 (75.9)			
Pregnancy status						
yes	22	6 (27.3)	16 (72.7)	0.19	1	0.80
no	84	27 (32.1)	57 (67.9)			
Age						
5-7 years	19	7 (36.8)	12 (63.2)	0.39	2	0.83
>7-10 years	55	16 (29.1)	39 (70.9)			
>10 years	32	10 (31.2)	22 (68.8)			
Location						
east	86	26 (30.2)	60 (69.8)	0.17	1	0.79
west	20	7 (35)	13 (65)			
Anti-sucking use						
yes	87	24 (27.6)	63 (72.4)	2.85	1	0.11
no	19	9 (47.4)	10 (52.6)			
Bacteria isolated						
yes	47	14 (29.8)	33 (70.2)	0.07	1	0.84
no	59	19 (32.2)	40 (67.8)			

Note: No.: the total numbers of examined camels, df: degree of freedom for Chi-square

Agab and Abbas (1999) suggested that the udder is a predilection site to ticks infestation resulting in skin and teat lesions, and consequently facilitating bacterial entry and leaving behind permanent tissue damages. This suggestion was in agreement with our finding in this study.

The results In this study showed all animal examined were infested with ticks, Maha(2009) indicated that *Hyalomma dromedaries* (*H. dromedaries*) was the main ticks species infested camels (69.9%) in Butana followed by (7.27%) *H. rufiets* and (6.38%) *H. impeltatum* However,

Amplomyomma lepidum (*A. lepidum*), *A. farigatum*, *Boophilis decoloratus* (*B. decoloratus*) and *Rhipicephalus sanguinus* (*R. sanguinus*) also were reported in a few number. The result also concluded that there was no significant difference between number of ticks collected from male and she-camels, however, the female camels carried more ticks than male according to our observation. A similar finding was reported in cows and was considered to be due to failure in animal management, hormonal effects, pregnancy and lactation stress resulting in lowering the resistance of female to ticks infestation (Hassan, 1997; Maha, 2009). The prevalence of mastitis was significantly affected by tick's infestation, similar suggestion also reported by other (Abera *et al.*, 2010, Obeid 1983).

In comparison it was reported that the prevalence of tick infestation in Ethiopia was 72% and the prevalence of mastitis was (30%) in heavily infested female camels compared with non-infested udder (9%). Other conditions include traumatic lesions and lacerations (Almaw and Mulla, 2000). The study suggested that the udder skin abscesses could be due to severe infestation with ticks and bite of insects larvae which may induce chronic mastitis or blocking of the teat canal. This suggestion was also reported by other investigators (Tibary and Anoassi, 2000).

This study noted that 124(82.7) cause used an anti-suckling device and that may cause teat canal obstruction leading to dilatation of the ducts, retention of

milk and secondary bacterial infections, similar observation was mention by Ramadan *et al.*,(1987).

In Kordofan state, 96.61% of the camel owners used anti-suckling devices and 85.05% of them claimed that the presence of anti-suckling devices without milking of lactating she-camel is the main causes of mastitis (Ismail, 2006). A clear relationship was observed when anti-suckling devices was used and occurrence mastitis and it incriminated as a risk factor in spreading of intra-mammary infections (Abdel-Gadir *et al.*, 2005). It was also mentioned that there was a positive correlation between teat lesions and the occurrence of sub-Clinical mastitis (Mulei, 1999).

Mammary neoplasia is rare in Camelids, the occurrence of carcinoma in mixed mammary tumor was identified by Bangara and Stevenson (2007) for the first time in a 13-year-old female *llama*. histopathological examination revealed tubulopapillary acinar or solid nest-like clusters of neoplastic epithelial cells surrounded by whorls and sheets of proliferation of epithelial cells and classified as a mixed tumor. Positive confirmed diagnosis was performed by Immuno-histochemical staining of neoplastic epithelial cells with anti-cytokeratin antibodies; metastasis to regional lymph nodes was not reported (Bangara and Stevenson, 2007).

In the current study five types of tumors were described viz. Intra-cystic papillary carcinoma, mixed mammary tumors (papillary carcinoma and fibroadenoma), adenocarcinoma, cystic adenomatous

hyperplasia and skin papilloma this was in agreement with Hegazy *et al*, (2004).

CONCLUSION:

The prevalence of mastitis in slaughtered female camels in Tumbool abattoir obtained by this study was relatively high at 29.7%. *Staphylococcus* spp. and *Streptococcus* spp. were the most commonly bacteria involved in the udder mastitis. Although we are unable to address the significance of the tick infestation with the isolated bacteria, all udders investigated were infested with ticks. We suggest that Anti-suckling device practice by camel owners have a role in udder affections. Chronic mastitis was the most prevalent type and hard udder texture was associated with the chronic phenomena of the disease. To our knowledge this is the first time that the tumor cases reported in the field and found to be a problem in the udder of the camels (30%). In conclusion, mastitis in camel is prevalent in the country and can be caused by different pathogens of bacteria. Despite the insignificance association between the examined risk factors and the neoplastic masses revealed by this study. Further research should be tackled in order to determine the role of the neoplastic masses in the disease prevalence for better epidemiological consideration.

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