Vol. 13(2), pp. 74-83, April-June 2021
DOI: 10.5897/JVMAH2020.0877
Article Number: D9DE89066602
ISSN: 2141-2529

http://www.academicjournals.org/JVMAH

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Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Bacteriology and cytology of the non-gravid one-humped camel genitalia

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Received 7 September, 2020; Accepted 25 January, 2021

This study evaluates the cytology and prevalence of bacteria in the genital tract of non-pregnant dromedary camels at the main abattoir of Kano through a cross-sectional design with convenience sampling. Results revealed that *Proteus mirabilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were isolated with *E. coli and S. aureus* being the most prevalent. These bacteria were more prevalent in the vagina followed by the cervix and were more sensitive to cefoxitin, gentamicin and amoxicillin. The vagina had more cell counts than other regions of the non-pregnant genitalia. No association was recorded between cell type and region of non-pregnant genitalia. Similarly, there was no association between cell morphology and region of non-pregnant genitalia. However, there was association between background content and region of non-pregnant genitalia. In conclusion, bacteria inhabiting the non-pregnant camelid genitalia are *Proteus* spp., *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with *E. coli and S. aureus* being the most prevalent. These were sensitive to cefoxitin, gentamicin and amoxicillin. The vagina compared to other regions of the genitalia had more cell counts. The background content was dependent on region of non-pregnant camel genitalia.

Key words: Bacteriology, cytology, one-humped camel, genitalia, antimicrobial sensitivity.

INTRODUCTION

The camel is an important species uniquely adapted to hot and arid environments (Schwartz, 1992), and as such contributes significantly to the food security of nomadic pastoral households (Faye, 1997). This unique adaptability makes the species ideal for exploitation under the arid and semi-arid land conditions (Bengoumi and Faye, 2002). The fragile relationship of people, livestock and environment is being upset by climate change (Lioubimtseva and Henebry, 2009). This climate change (Global warming) has increased the frequency and severity of drought in marginal areas, such as the semi-arid and arid/desert areas (Sirohi and Michaelowa, 2007). Recent droughts have resulted in all households losing livestock, especially donkeys, cattle, sheep and

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goats, which are less hardy than camels and this has increased the prevalence of hunger (Tschakert, 2007). Most pastoralists realize that, in order to survive the new reality of more frequent drought, they need more camels because they are hardier and can graze on shrubs and trees that other livestock cannot (Faye and Bonnet, 2012).

The camel is a domesticated animal whose full agricultural potential has not yet been explored (Mohammed, 2000). For camel to achieve greater significance, its traditional forms of husbandry have to be modified, and this will require a better understanding of the basic biology of the camel and its potentials (Skidmore, 2000). This will include an understanding of its genetics, reproduction and nutrition which hold the key to the successful utilization of the camel as a growing protein source (Skidmore, 2003; Bello et al., 2012).

Poor reproductive efficiency has been described as a major problem in camelids (Bello et al., 2012). In the camel, the reproductive rate varies between 25 and 80% depending on levels of management and veterinary care (Tibary and Anouassi, 1997a). Various reproductive disorders, especially uterine infections, have been described in camelids and may play an important role in reduced fertility in these species (Tibary and Anouassi, 1997b). Like in many domestic animal species, uterine infections are the most common disorders in camelids (Nur, 1984; Johnson, 1989; Wernery and Wernery, 1992; Wernery and Kumar, 1994; Tibary and Anouassi, 1997b; Fowler, 1998; Tibary and Anouassi, 2000), but unlike other species, little is known about their pathogenesis and evolution.

Cytological evaluation of reproductive status has been used for bitch and mare (Noakes et al., 2001) and cow (Raab et al., 2002; Kasimanickam et al., 2004). Cytological examination of the endometrium is often used in the mare to evaluate causes of infertility, specifically to detect venereal disease and acute or chronic endometritis (Slusher et al., 1992; Ricketts et al., 1993). There is no much in-depth study on the reproductive cytology of camels, as such camel reproductive disorders and infections are treated based on findings in other domestic animals such as cattle, horses etc. Studies on cytology and bacteriology of the camel reproductive tract are necessary for understanding of camels' cytological dynamics to facilitate identification of specific medical interventions to handle their reproductive disorders and infections.

Opportunist infections with a variety of bacteria are more important causes of endometritis worldwide and significantly affect fertility (Henderson, 1990). The importance of studying such microorganisms is related to diseases caused by them due to stress and reduction of the immunity of the reproductive system (Al-Dahash and Fathalla, 2000). The aim of this study is, therefore, to determine the prevalence of bacteria and cytological characteristics of the non-pregnant camel genitalia.

MATERIALS AND METHODS

Sampling location and study animals

Samples for this study were collected from Kano main abattoir. The types of animals slaughtered in the abattoir are mainly ruminants, which include cattle, camels, sheep and goats. The abattoir operates every day of the week and is the main supplier of meat to the highly populated metropolitan city of Kano (2,828,861 people - 2006 census) and its environs. The camels used for this study originated from the extreme northern part of Nigeria and neighbouring Sub-Saharan countries such as Niger, Chad, and Sudan etc. They were culled from pastoral herds using natural breeding and their parity and other reproductive histories were unknown.

Study design and sample size determination

A cross-sectional study design with convenience sampling technique was employed for samples collection from camels at Kano main abattoir. The sample size used by Shokri et al. (2010) was adopted with modifications. In this regard, complete genital tracts from 50 non-pregnant dromedary camels were utilized for the study. For each camel, two swab samples each were collected from the vagina, cervix, uterine body and uterine horns (left and right), making a total of 500 swab samples. Thus, 2 swab samples each from 5 organs within 50 genital tracts.

Data collection

The slaughter slab phase

Female camels aged 4 years and above were tagged. After slaughter, only those without fetus *in utero* on evisceration were considered for the study. The complete genital tract was removed aseptically, ligated with a nylon suture and transported under cool condition to the Microbiology laboratory of the Aminu Kano Teaching Hospital (AKTH), Kano.

Laboratory phase

Collection of swab samples: A longitudinal incision from the outer vaginal surface through the entire length of the genital tract was made using a sterile scalpel blade and both edges of the incision were opened using sterile forceps. At the level of the uterine body another incision was made in the direction of the two uterine horns. Swab samples were collected according to a modification of the procedure of (Cocchia et al., 2012). Briefly, the tip of the two swab sticks was made wet with few drops of sterile normal saline and rolled onto the vaginal mucosal surface for 15 s. The swab tips were placed against the mucosa to soak up the secretions for another 15 s. One out of the two swab sticks was then rolled onto a sterile microscope slide, air dried for 30 to 35 min, fixed in 96% ethanol and submitted for cytology. The other was submitted for bacteriology within 3 h. The same procedure was repeated for the cervix, uterine body and left and right uterine horns.

Bacteriological culture, isolation and identification

Swabs were cultured on Chocolate, Sabouraud and MacConkey agar and incubated at 37°C for 24 to 48 h. Standard biochemical

Table 1. Isolation pattern of bacteria from regions of non-pregnant female camel genitalia.

Reproductive region	Without growth	With growth	Total frequency of isolates
Right Uterine Horn	48 (96.00%)	2 (4.00%)	2 (6.06%)
Left Uterine Horn	50 (100.00%)	0 (0.00%)	0 (0.0%)
Uterine Body	50 (100.00%)	0 (0.00%)	0 (0.0%)
Cervix	42 (84.00%)	9* (16.00%)	9 (27.27%)
Vagina	30 (60.00%)	22** (40.00%)	22 (66.67%)
Total	220 (88.00%)	33 (12.00%)	33

*One swab with double isolates; **two swabs with double isolates.

tests were used for the identification of isolates (Quinn et al., 1994; Cowan and Steel, 2004; Koneman et al., 2005).

Antimicrobial sensitivity of bacterial isolates

The antimicrobial agents tested were as follows:

(1) Cefoxitin
(2) Amoxycillin
(3) Gentamycin
(4) Penicillin
(5) Neomycin
(6) Streptomycin

The sensitivity to the above-mentioned antibiotics was determined using Kirby-Bauer procedure as described by Demissie (2011).

Cytological examination

The slide fixed in 96% ethanol above was stained with Papanicolaou stain within two hours of arrival at the laboratory. It was then examined for total cellularity, cell morphology and background content according to a procedure of Cocchia et al. (2012) with modifications. Briefly, the cells were classified as endometrial epithelial cells, neutrophils and other inflammatory (eosinophils, lymphocytes or macrophages) or epithelial cells. The background content was assessed as proteinaceous, contaminated with red blood cells or clear. Quality of cells harvested was recorded as intact, distorted or fragmented. Cellularity was assessed as number of cells per high powered field (HPF). The percentage of distorted epithelial cells was measured in 10 HPF and averaged. The number of polymorphonuclear neutrophils was counted in 10 fields and result expressed as a percentage of the total number of cells in 10 HPF.

Data analysis

Descriptive statistics was used in analyzing data on the genital bacteria and their isolation rates (prevalence) in the non-pregnant camels. The comparison of inflammatory cell types among vagina, cervix, uterine body and uterine horn smears was done by Fisher's Exact test or Chi-Square test for independence as the case may be. Unless otherwise stated, P-value was considered significant at ≤ 0.05. All statistical tests were performed using the Statistical software SPSS (Statistical Package for the Social Sciences version 21.0, SPSS Inc., Armonk, NY, USA).

RESULTS

Bacteria prevalence within the non-pregnant camel genitalia and antimicrobial sensitivity pattern of the bacteria isolates

The isolated types of bacteria from the non-pregnant reproductive tract of female camels are presented in Table 1. Bacteria were isolated from 30 of all the swabs in the study. The prevalence of bacteria was highest in the vagina, followed by the cervix and the right uterine horn. No bacteria were isolated from the right uterine horn and the uterine body of the animals. The distribution of bacterial isolates according to Gram stain reaction is highlighted in Table 2. Out of all the bacterial isolates, Gram negative bacteria were the most detected. The vagina recorded the highest Gram negative bacterial isolates.

The biochemical identification of bacterial isolates from female camel genitalia is presented in Table 3. Six types of bacteria were identified among the bacterial isolates recorded. The identified types of bacteria were *Proteus mirabilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The distribution of bacterial species across non-pregnant camel genitalia is presented in Table 4. Six types of bacteria were identified. The isolation rate of *Escherichia coli* was higher, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. The vagina recorded the highest isolation rate where all the six types of bacteria were isolated with *Staphylococcus aureus* having the highest isolation rate. However, in the cervix 5 types of bacteria were isolated, where *Escherichia coli* had the highest isolation rate, followed by *Staphylococcus aureus* and the others (*Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*). In the right uterine horn, only

Table 2. Distribution of bacteria detected based on Gram stain reaction (direct smear) from the different reproductive regions of the camel genitalia.

Reproductive region	Gram +ve	Gram –ve	Total
Right Uterine Horn	0 (0.0%)	2 (100%)	2
Left Uterine Horn	0 (0.0%)	0 (0.0%)	0
Uterine Body	0 (0.0%)	0 (0.0%)	0
Cervix	2 (22.2%)	7 (77.7%)	9
Vagina	7 (31.8%)	15 (68.2%)	22
Total	9 (27.3%)	24 (72.7%)	33

Table 3. Biochemical identification of bacterial isolates from non-pregnant female camel genitalia.

Bacterial Isolates	Biochemical test						
	Urease	Citrate	Indole	Kligler's iron agar (KIA)			
				Slope	Butt	H_2S	Gas
Proteus mirabilis	+	+	–	R	Y	+	+
Proteus vulgaris	+	D	+	R	Y	+	d
Klebsiella pneumoniae	+	+	–	Y	Y	–	+
Escherichia coli	–	–	+	Y	Y	–	+
Pseudomonas aeruginosa	Oxidase +						
Staphylococcus aureus	Catalase +			Coagulase +			

H2S = Hydrogen sulphide (blackening), R = Red-pink (alkaline reaction), Y = Yellow (acid reaction), d = different strains give different results.

Table 4. Distribution of bacterial species according to regions of the non-pregnant camel genitalia.

Bacterial species	Isolation rate	Regional isolation rate				
		RUH	LUH	UB	Cervix	Vagina
Proteus mirabilis	2 (6.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (9.1%)
Proteus vulgaris	4 (12.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (11.1%)	3 (13.6%)
Klebsiella pneumoniae	4 (12.1%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	1 (11.1%)	2 (9.1%)
Escherichia coli	11 (33.3%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	4 (44.4%)	6 (27.3%)
Pseudomonas aeruginosa	3 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (11.1%)	2 (9.1%)
Staphylococcus aureus	9 (27.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (22.1%)	7 (31.8%)
Total	33	2 (6.1%)	0 (0.0%)	0 (0.0%)	9 (27.3%)	22 (66.7%)

RUH = Right Uterine Horn, LUH = Left Uterine Horn, UB = Uterine Body.

2 types of bacteria were isolated; *Escherichia coli* and *Klebsiella pneumoniae*. No bacteria were isolated from the left uterine horn and the uterine body.

The sensitivity pattern of the bacterial isolates to antibiotics is shown in Table 5. The result of the sensitivity pattern of all the bacterial isolates indicated that gentamycin, cefoxitin and amoxicillin recorded higher sensitivity, followed by penicillin and neomycin. Streptomycin had the lowest sensitivity. Summary statistics (including KW statistic) for median cell count among the right and left uterine horn, uterine body, cervix and vagina of the non-pregnant camel genitalia are presented in Table 6. The left uterine horn recorded the highest cell count (13) followed by the uterine body (10), vagina (9) and then the cervix (8). Conversely, the right uterine horn recorded the lowest cell count (5) when compared to the other regions of the genitalia.

Dunn's Multiple Comparisons Test for cell count among

Table 5. Antimicrobial susceptibility pattern of bacterial isolates from the non-pregnant camel genitalia.

Bacterial isolates	Frequency of isolation	Antibiotic susceptibility of isolates					
		FOX	AML	CN	P	N	S
Proteus mirabilis	2 (6.1%)	2 (100%)	1 (50.0%)	2 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Staphylococcus aureus	9 (27.3%)	8 (88.9%)	7 (77.8%)	8 (88.9%)	7 (77.8%)	5 (55.6%)	3 (33.3%)
Proteus vulgaris	4 (12.1%)	2 (50.0%)	3 (75.0%)	4 (100%)	1 (25.0%)	3 (75.0%)	4 (100%)
Pseudomonas aeruginosa	3 (9.1%)	0 (0.0%)	3 (100.0%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Escherichia coli	11 (33.3%)	9 (81.8%)	8 (72.3%)	8 (72.3%)	2 (18.2%)	2 (18.2%)	2 (18.2%)
Klebsiella pneumoniae	4 (12.1%)	3 (75.0%)	1 (25.0%)	4 (100%)	1 (25.0%)	1 (25.0%)	1 (25.0%)
Total Isolation	33	24	23	27	11	11	10
% Sensitivity		72.7	69.7	81.8	33.3	33.3	30.3

FOX = Cefoxitin, AML = Amoxicillin, CN = Gentamycin, P = Penicillin, N = Neomycin, S = Streptomycin.

Table 6. Summary statistics (including KW statistic) for cell count among regions of non-pregnant camel genitalia.

Region	N	Median	Minimum	Maximum	Sum of ranks	Mean of ranks
RUH	45	5	1	40	3846.0	85.47
LUH	50	13	1	44	7096.0	141.92
UB	47	10	1	68	6581.5	140.03
Cervix	49	8	1	32	5575.0	113.78
Vagina	49	9	1	50	5821.5	118.81

RUH = Right Uterine Horn, LUH = Left Uterine Horn, UB = Uterine Body, Kruskal-Wallis (KW) statistic = 20.486 (corrected for ties), $P<0.01$.

Table 7. Dunn's multiple comparisons test for cell count among regions of non-pregnant camel genitalia.

Comparison	Mean rank difference	P-value
RUH vs. LUH	-5.031	P>0.05
RUH vs. UB	26.256	P>0.05
RUH vs. Cervix	28.144	P>0.05
RUH vs. Vagina	-28.309	P>0.05
LUH vs. UB	21.226	P>0.05
LUH vs. Cervix	23.114	P>0.05
LUH vs. Vagina	-33.339	P>0.05
UB vs. Cervix	1.888	P>0.05
UB vs. Vagina	-54.565	P<0.01
Cervix vs. Vagina	-56.453	P<0.01

RUH = Right Uterine Horn, LUH = Left Uterine Horn, UB = Uterine Body.

the right and left uterine horn, uterine body, cervix and vagina of the non-pregnant camel genitalia is presented in Table 7. There was a statistically significant ($P<0.01$) difference in cell count between uterine body and vagina. Similarly, the cell count differed significantly ($P<0.01$) between cervix and vagina. However, there was no significant ($P>0.05$) difference in cell count between the right uterine horn and left uterine horn, right uterine horn and uterine body, right uterine horn and cervix, right uterine horn and vagina, left uterine horn and uterine

Table 8. Association between cell type and region of non-pregnant camel genitalia.

Cell type	Region of genitalia					Total
	RUH	LUH	UB	Cervix	Vagina	
Neutrophil	6	14	12	23	19	74
Lymphocyte	8	9	11	12	13	53
Epithelial cell	45	46	43	49	38	221
Total	59	69	66	84	70	348

RUH = Right Uterine Horn, LUH = Left Uterine Horn, UB = Uterine Body, χ^2 = 10.275, P>0.05.

Table 9. Association between cell morphology and region of non-pregnant camel genitalia.

Cell morphology	Region of genitalia					Total
	RUH	LUH	UB	Cervix	Vagina	
Intact	32	37	41	46	36	192
Distorted	45	47	46	49	39	226
Fragmented	27	32	30	26	33	148
Total	104	116	117	121	108	566

RUH = Right Uterine Horn, LUH = Left Uterine Horn, UB = Uterine Body, χ^2 = 3.725, P>0.05.

Table 10. Association between background content and region of non-pregnant camel genitalia.

Background content	Region of genitalia					Total
	RUH	LUH	UB	Cervix	Vagina	
Clear	18	18	28	13	7	84
Red Blood Cells	29	26	16	26	39	136
Proteinaceous	13	16	11	26	22	88
Total	60	60	55	65	68	308

RUH = Right Uterine Horn, LUH = Left Uterine Horn, UB = Uterine Body, χ^2 = 31.855, P<0.01.

body, left uterine horn and cervix, left uterine horn and vagina, and uterine body and cervix.

The association between cell type and region of non-pregnant camel genitalia is presented in Table 8. There was no statistically significant association (χ^2 = 10.275, P>0.05) between cell type and region of non-pregnant camel genitalia. The association between cell morphology and region of non-pregnant camel genitalia is presented in Table 9. The result showed no statistically significant association (χ^2 = 3.725, P>0.05) between cell morphology and region of non-pregnant camel genitalia. The association between background content and region of non-pregnant genitalia is shown in Table 10. There was significant association (χ^2 = 31.855, P<0.01) between background content and region of non-pregnant camel genitalia. Background content with red blood cells was highest (136), followed by proteinaceous (88) across regions of the non-pregnant camel genitalia. However, clear background content was the lowest (84) within the reproductive regions of the non-pregnant camelid genitalia. The result showed background content with red blood cells to be highest in the vagina. Proteinaceous background content was found to be highest in cervix while the clear background content recorded highest occurrence in the uterine body.

DISCUSSION

Bacterial prevalence

During the reproductive life of a female camel the reproductive tract is exposed to the risks of infection, particularly at the time of breeding and following

parturition when various microorganisms are carried from the environment and the posterior part of the genitalia (Vagina) into the interior part of the reproductive tract (Tibary and Anouassi, 2001). Some of these micro-organisms are bacteria which inhabit the reproductive tract of the female camel (*Camelus dromedarius*) and have been shown to be highly responsible for reproductive disorders in this species (Tibary et al., 2006; Ali et al., 2010a). Information regarding bacteria causing genital infections in female dromedary camels is scarce (Ali et al., 2010a). Bacteria are regarded as the most important cause of infertility in domestic species (Simenew et al., 2015). In the current investigation *Proteus mirabilis*, *P. vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* constituted the microflora of the genitalia of the non-pregnant camel. Animals from which these organisms were isolated are those bred majorly using natural mating which is characterized by low reproductive performance, mostly associated with puerperal infections of the genital tracts (Sheldon et al., 2006). In *Camelidae*, during mating, the penis penetrates the cervical canal and enters deep into the uterine cavity (Vaughan and Tibary, 2006). Repeated insults of the female genitalia due to improper mating practices can lead to inflammation and loss of the ability to resist infection (Tibary et al., 2006). Chronic vaginitis, overbreeding, aggressive mating practice, injuries during parturition and increased parity could be suggested as factors which contribute to fertility problem. Moreover, some herdsmen place unusual substances, like dates, black seeds and salts in the vagina of animals with fertility problems as part of ethno-veterinary practice (Ali et al., 2010b). Evidence implicating bacterial infections as causes of endometritis has been reported (Mshelia et al., 2014), and a variety of these bacterial species have been recovered from the uteri of infertile camelids (Wernery and Kumar, 1994; Tibary et al., 2006). The most common bacteria isolated within genitalia of camelids with endometritis were *Escherichia coli, Proteus* spp*.* and *Klebsiella pneumoniae* (Wernery and Wernery, 1992; Wernery and Kumar, 1994). *Escherichia coli* has been isolated from cases with purulent vaginal discharges in female camels (Nielsen et al., 2010). It was reported that *E. coli* was more associated with repeat breeding than with clinical endometritis (Knudsen, 1982). *E. coli* and *Proteus* spp. have been considered in equine and cattle as nonspecific pathogens associated with endometritis (Couto and Hughes, 1984) and they were observed in cases with purulent discharges (Nielsen et al., 2010). *Pseudomonas aeruginosa* have been isolated from infertile camels and may be associated with venereal transmission and should be considered in infertility or abortion outbreaks (Wernery and Wernery, 1992). *Staphylococcus aureus*, *E. coli* as well as *Proteus* spp. were frequently isolated from female camels with uterine infections (Ali et al., 2010b). This study identified *Staphylococcus aureus* and *E. coli* as the most common bacteria isolated which agrees with findings of Ali et al. (2010b) who reported these bacteria as the most isolated in female reproductive tract of camels in Saudi Arabia.

Vagina being the organ that connects the reproductive tract to the external environment and through which intromission does occur during coitus is exposed to a variety of bacteria. These bacteria might be from the environment or from the male reproductive organ during coitus. The vagina is constantly being contaminated with these bacteria from the environment and from faecal droppings that smear the vagina during breeding. These and other contaminants from the male genitalia are introduced into the female vagina during coitus (Tibary and Anouassi, 2001; Singh et al., 2008). The vagina in the current study recorded all six types of bacteria (*Proteus mirabilis*, *P. vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) with *Staphylococcus aureus* being predominant followed by *Escherichia coli*. The same type of bacteria (*Proteus* spp., *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp. and *Staphylococcus aureus*) was reported to have been isolated with *Escherichia coli* being predominant from the vagina of multiparous non-pregnant cows presented for artificial insemination and it was attributed to primitive unhygienic housing and breeding of cows (Kather et al., 2012). Due to the hormonal changes in females at pre-breeding and peri-parturient periods, bacteria colonizing the vagina can ascend into the cervix, which opens during these periods, and enters the uterus to establish infections that lowers reproductive efficiency in animals (Couto and Hughes, 1984). Also in cows, during the immediate period post-partum the cervix is dilated (Sheldon and Dobson, 2004) which allows bacteria to ascend from the vagina into the uterus, causing infections in 90% of cows by 21 days post-partum (Sheldon et al., 2006). This might be the reason why in the present study five out of the six types of bacteria (*P. vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) isolated from the vagina were isolated from the cervix with *E. coli* being predominant. Isolation of similar bacteria was reported from the cervix of camels with *S. aureus* being predominant (Wernery and Ali, 1989).

Though very few bacteria were isolated from the uterus (right uterine horn) in this study when compared to the vagina and cervix, endometritis may still exist due to the fact that failure to isolate bacteria does not necessarily prove its absence (LeBlanc and McKinnon, 2011). Isolation of very few bacteria from the uterus might be attributed to the continuous clearance of bacteria from the uterine lumen (Couto and Hughes, 1984) by the natural uterine defense mechanisms (Tibary and Anouassi, 2001). Regardless of this low bacteria isolates in the uterus, the isolation of pathogenic bacteria such as *E. coli* in this study portends a risk factor for lowered

reproductive efficiency due to increased inflammatory reactions and possible damages to the uterine tissues by direct action of the bacteria or its toxins (Kather et al., 2012).

Antimicrobial sensitivity of bacterial isolates

Antimicrobial agents are used in the management of reproductive failures in livestock (Drillich, 2006). Antibiotics are commonly used in the treatment of reproductive tract infection in camels (Tibary and Anouassi, 2001). However, the efficacy of such therapeutic agents needs to be evaluated occasionally due to continuous emergence of drug resistant bacterial strains (Vekateswaran and Rjeswar, 1991). It has been observed that there is general global rise in antibiotic resistance linked to an increased use of antibiotics (Swartz, 1997). In artificial insemination practice antibiotics have long been used both prophylactically before breeding, as a treatment for endometritis as well as in semen extenders (Albihn et al., 2003). As components of semen extenders, antimicrobial agents are used in order to inhibit growth of bacteria in semen for artificial insemination (El-Bahrawy et al., 2010). Therefore, the difference in antibiotic susceptibility might depend on the way an antimicrobial agent is used (Albihn et al., 2003).

The present study revealed that *Staphylococcus aureus* was among the bacteria predominantly isolated from the genitalia of non-pregnant camels and was sensitive to most of the antimicrobial agents tested which include cefoxitin, gentamicin, amoxicillin, penicillin and neomycin. These findings concur with the observations made by Fazlani et al. (2011) and Mshelia et al. (2014) that *Staphylococcus aureus* was sensitive to most of the antimicrobial agents tested among which was amoxicillin. Also, Teshome et al. (2016) reported that susceptibility patterns of *S. aureus* to antimicrobial agents varied worldwide, but isolates were usually susceptible to some antibiotics, among which were cefoxitin and gentamicin. *S. aureus* is a common human and animal pathogen and a usual cause of invasive and life threatening infections (Teshome et al., 2016). *S. aureus* has also been found to be a major causative pathogen of clinical and subclinical mastitis in animals (Adwan et al., 2005; Mekuria et al., 2013).

Escherichia coli was reported to be a non-specific pathogen found in mares and cows with endometritis (Arthur et al., 2000), and isolated in camels with purulent discharges (Ali et al., 2010b). In this study, *E. coli* was found to be sensitive to cefoxitin, gentamicin and amoxicillin. Ali et al. (2010b) reported *E. coli* to be sensitive to gentamicin.

Klebsiella pneumoniae, *Proteus* spp. and *Pseudomonas aeruginosa* have been classified as non-specific pathogens that are associated with endometritis (Arthur et al., 1985) and have been isolated from camels with vaginal purulent discharges (Ali et al., 2010b). The present study revealed *K. pneumoniae* to be sensitive to cefoxitin and gentamicin, Proteus *mirabilis* was sensitive to cefoxitin, gentamicin and amoxicillin and *P. aeruginosa* had sensitivity to only amoxicillin. *Proteus vulgaris* was sensitive to cefoxitin, gentamicin, amoxicillin, neomycin and streptomycin.

Cytology of the non-pregnant camelid genitalia

Local immunity, phagocytosis and mechanical clearance by myometrial contractions are the major mechanisms used to clear uterine infection, and are more effective during the follicular phase of the oestrous cycle, when oestrogens are high and the uterus has maximal contraction (Tibary and Anouassi, 1997b). Failure of these defence mechanisms leads to the establishment of a uterine infection and the development of an endometritis or metritis, and usually occurs when uterine resistance is diminished due to degenerative changes in the endometrium (fibrosis) or repeated heavy infection with pathogenic microorganisms (Tibary and Anouassi, 2001).

Cytological evaluation of the entire or part (endometrium) of the reproductive tract is used as a diagnostic tool for detection of venereal disease, uterine infections and acute, chronic or subclinical endometritis in mares (Slusher et al., 1992; Ricketts et al., 1993: Noakes et al., 2001) cow (Raab et al., 2002; Kasimanickam et al., 2004) and camels (Wernery and Kaaden 2002).

In a cytological study of bovine cervical mucosa and endometrium, Ahmadi et al. (2005a) observed no significant difference in cellular density between the organs. Similarly, Ahmadi et al. (2005b) reported no significant difference between cervical and uterine cytological evaluation in dromedary camels and opined that cytological evaluation of the cervix can be used for screening of camels for reproductive status, especially during postpartum periods. However, the present study revealed that cellular density in the vagina was found to be significantly higher than corresponding counts in both the cervix and uterine body. But, the study found no significant association between region and cell type, and also between region and cell morphology of the non-pregnant camel genitalia. The degree of inflammation is assessed by an evaluation of the number and the morphology of polymorphonuclear (PMNs) leucocytes and presence of three to five PMNs per high power field is usually significant in the diagnosis of endometritis (Tibary and Anouassi, 2001). Ahmadi et al. (2000) reported significant differences in the mean percentages of neutrophils in cervical mucosa smears at different oestrous cycle phases (days 0, 2, 10 and 19) of synchronized heifers. Histological investigations of the uterus have been performed on camelids by various

scientists in connection with the follicular waves (Fowler, 1998; Beil, 2002). Since camelids do not cycle as most animals do, histological parameters associated with the stages of oestrous cycle have not been described.

Conclusion

It can be concluded that the bacteria inhabiting the camelid genitalia are *Proteus* spp, *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *S. aureus* with *E. coli and S. aureus* being the most prevalent. These bacteria were sensitive to cefoxitin, gentamicin and amoxicillin. The vagina had more cell counts than other regions of the non-pregnant genitalia. Background content of samples for cytology was dependent on regions of non-pregnant camel genitalia from which they were obtained.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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