

Kinetics of the Uterine Bacterial and Hormonal in She-Camel, (*Camelus Dromedaries*), During the Postpartum

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Abstract: The normal uterine flora is dominated by lactobacilli which play a vital role in maintaining uterine health. During the part, the uterus is exposed to exogenous and endogenous infections causing a change in the uterine flora. In she-camels, the composition of the uterine microbiota after parturition remains poorly documented. The aim of this study was to investigate the kinetics of uterine bacterial flora after calving coupled with hormonal dosage. Uterine swabs and blood samples were collected at 8, 15, 21, 28, 35, 42 days after the part. A total of 354 Gram-positive bacteria were identified, the catalase test showed that 80.2% were *Staphylococci* and 19.8% were *Streptococci*. Identification on the API galleries showed two *Staphylococci* (*S. lentus* and *S. xylosus*) and 15 *Streptococci* including 26.1% of *Aerococcus* (*A. viridans* 1, 2 and 3), 30.68% of *Streptococcus* (*S. pyogenes*, *S. dysgalactiae* ssp. *Equisimilis*, *S. pneumoniae*, *S. bovis*, *S. uberis*, *S. mitis* 1), 13.02% of *Enterococcus* (*E. faecium*, *E. faecalis*, *E. avium*), 8.4% of *Leuconostoc* spp. and 21.60% of *Lactococcus* (*L. lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*). Progesterone has a positive effect on the proliferation of *Staphylococci* and *Streptococci* ($p < 0.05-0.001$) while 17 β Oestradiol has a negative effect ($p < 0.05$). These last homo-fermented acidophilic species which produce lactic acid, seem to strengthen uterine defence during the postpartum period in she-camels.

Key words: Bacteria, camel, oestrogens, progesterone, uterine swabs, homo-fermented

INTRODUCTION

The dromedary "*Camelus dromedarius*" is a species naturally adapted to the desert and Saharan regions. He is able to overcome the global warming which leads to desertification and drying up of the lakes, threatening all the natural ecosystems of the planet (Ali *et al.*, 2012; Reiter, 2001).

According to Kelanemer *et al.* (2015), the 4/5 of the Algerian territory are characterized by an arid and semi-arid climate where live 31 5000 camels, this population contributes to the production of milk and meat intended for nomadic population, this genetic resource remains lower compared to the production capacities of the country. The improvement of the farming system will help to increase the production of milk and meat.

In dromedary, the sexual cycle is follicular and the corpus luteum is formed after induced ovulation, the uterine involution is complete between 15-21 days (Skidmore, 2011; Derar *et al.*, 2014). Outside pregnancy,

progesterone remains below 1 ng/mL and an oestrogen ranges from 25-51 pg/mL (Kelanemer *et al.*, 2015). The placenta of camel is diffuse, calving occurs spontaneously and placental retentions are rare (Sghiri and Driancourt, 1999). Nevertheless, the uterus remains at risk of infections during and after calving. The literature review showed that uterine infections occur in female dromedary, the studies were performed on females with metritis and endometritis or on post-slaughter dams (Ali *et al.*, 2010). The main isolated germs are *Staphylococci aureus*, *Streptococci pyogenes*, *Echerichia coli*, *Corynebacterium pyogenes*, *Corynebacterium* spp. and Coliform species (Mshelia *et al.*, 2012; Nabih and Osman, 2012). These pathogens are responsible for severe uterine infections and the frequency of these infections increases with cervical and vaginal adhesions and prolonged progesterone treatments (Tibary and Anouassi, 2001).

The diagnosis of uterine infections is very important for the prevention of risks of infertility or even sterility;

these infections are dependent on the balance between the contaminating germs and the local immune defence of the uterus. In the later, we note the inflammatory responses, the high levels of oestrogen that stimulates the uterine motility, cervical opening and fluid elimination (Wulster-Radcliffe *et al.*, 2003). The uterine infection is more important under the influence of progesterone than oestrogen (Lewis, 2004).

Studies on the uterine microbiota during the postpartum period in she-camel are rare. In order to complete the physiological part, related to the resumption of ovarian activity in she-camel from the birth to the first heat. It seems appropriate to carry out investigations on the evolution of bacterial flora in the camel after calving. To achieve these objectives, we followed bacterial activities coupled to hormonal kinetics after uterine involution in 10 she-camels, from the 1st to the 6th week after parturition.

MATERIALS AND METHODS

The study was conducted with 10 camels of Saharawi breeds aged 10-12 years. Females were followed for 6 weeks after natural calving at 1 week intervals. Before handling, each female receives an intravenous injection of 8-10 mL of Xylazine® 20% per animal (KEPRO Holland) (Vyas *et al.*, 2008).

After tranquilization of the female using a bovine type vaginoscope (Tibary and Anaouassi, 2001), uterine swabs were collected at 8, 15, 21, 28, 35, 42 days after the part with sterile disposable swabs (Equivet 663021) and placed in a transport medium (nutrient broth) under cold conditions and immediately sent to the laboratory for bacterial culture.

The previously collected swabs were incubated for 24 h at 37°C; the samples were inoculated on different culture media. Nutrient agar has been used to grow non-demanding bacteria. The bacteria obtained were stained with Gram and the catalase test. The detection of *Streptococci* was done on blood agar. Selection of *Staphylococci* was done in Chapman agar. The growth of coliforms was carried out on the desoxycholate agar (Hogan *et al.*, 1999).

The biochemical identification of the isolated strains was made using API 20 staph, API 20 strep and API 20 E (Bio Merieux) and the identification was performed using Apiweb™ Software, available on the website Merieux, <https://apiweb.Biomerieux.com/servlet/>.

Simultaneously to the swab collection, blood samples were taken on dry tubes at the same time after the part and the determination of 17 β-estradiol and progesterone was carried out by the Microparticulate Enzyme Immunoassay

method (MEIA) and the AxSYM (progesterone MAIA®). The basal detection rate of the technique is 10 µg/mL of serum for oestrogen and 0.1 ng/mL of serum for progesterone.

A descriptive statistical was performed on the data using SAS Software (Statistical Analysis System 2000). The fixed effect of the weeks and the random effect of the animal on the various measured parameters were done using the SAS software using the mixed procedure. The significance level used was p<0.05.

RESULTS AND DISCUSSION

Bacterial culture counted 354 Gram-positive bacteria. Of those isolated on blood agar, 33.8% is haemolytic and 66.2% non-haemolytic. In contrast, colonies of coliforms were not recorded. The catalase susceptibility test showed that 80.2% is catalase positive *Staphylococci* and 19.8% is catalase negative *Streptococci*. Table 1 shows the number of *Staphylococci* and *Streptococci* identified in each camel during 6 weeks of the postpartum. An individual variation in the number of *Staphylococci* and *Streptococci* was observed between the 10 camels (Table 1). Nevertheless, the number of *Staphylococci* in female 1 was higher (p<0.05) compared to female 9 (Table 1). And the female 9 showed a lower number of *Streptococci* compared to females 1, 5, 6, 7, 8 and 10 (p<0.05-0.001).

The kinetics of progesterone and 17 β-estradiol is highlighted in Table 2. The progesterone level is inversely proportional (p<0.05) to that of 17 β-estradiol (Table 2). There is a decrease in progesterone level from the first, until the third week after delivery followed by the increase of the kinetics of the hormone from the 4th to the 6th week postpartum (Table 2). The plasma concentration of 17 β-estradiol is lower during the first week after birth (Table 2), this rate increased gradually until the 4th week of the postpartum (Table 2) and the hormone decreased from the 5th-6th week after farrowing (Table 2).

Table 1: Number of *Staphylococci* and *Streptococci* identified in each female camel during the 6 weeks of the postpartum

Camels number	<i>Staphylococci</i>		<i>Streptococci</i>	
	Numbers	Percentage	Numbers	Percentage
1	33	7.62	8	1.98
2	26	8.19	4	2.26
3	30	7.34	6	2.26
4	30	7.34	5	2.26
5	32	10.16	10	1.98
6	31	7.90	9	2.26
7	29	8.19	8	1.98
8	28	7.90	11	1.98
9	20	7.34	3	1.41
10	25	8.19	6	1.41

Table 2: Relationship between hormonal parameters and number of *Staphylococci* during the 6 weeks of the post-partum

Weeks	<i>Staphylococci</i>		Progesterone (ng/mL)	17 β oestradiol (pg/mL)
	Number	Percentage		
1	76	21.5	0.78±0.17 ^{NS}	27.20±7.34 ^{NS}
2	46	12.7	0.29±0.17 ^{NS}	34.34±7.34*
3	26	7.3	0.18±0.17*	43.2±7.34 ^{NS}
4	36	10.2	0.22±0.17 ^{NS}	43.17±7.34 ^{NS}
5	45	12.7	0.26±0.17***	33.60±7.34 ^{NS}
6	56	15.8	0.45±0.17*	29.15±7.34 ^{NS}

NS: Not Significant*: Significant: p<0.05-0.01, ***Highly significant: p<0.001

Table 3: Relationship between hormonal parameters and number of *Streptococci* during the 6 weeks of the post-partum

Weeks	<i>Streptococci</i>		Progesterone (ng/mL)	17 β oestradiol (pg/mL)
	Number	Percentage		
1	18	5.09	0.78±0.17***	27.20±7.34 ^{NS}
2	13	3.67	0.29±0.17 ^{NS}	34.34±7.34 ^{NS}
3	6	1.69	0.18±0.17**	43.20±7.34**
4	6	1.69	0.22±0.17 ^{NS}	43.17±7.34 ^{NS}
5	10	2.82	0.26±0.17***	33.60±7.34 ^{NS}
6	17	4.80	0.45±0.17**	29.15±7.34 ^{NS}

NS: Not Significant*: Significant : p<0.05-0.01, ***Highly significant: p<0.001

The number of *Staphylococci* identified in the 10 camels during the 6 weeks of the postpartum is presented in Table 2. The number of *Staphylococci* was higher during the first week of the postpartum, followed by a decrease until the third week of the part. From the 4th week, we recorded an increase in the number of *Staphylococci* until the 6th week on the part (Table 2). There is no significant difference in the numbers of *Staphylococci* between the 6 weeks of study (p>0.05).

The number of *Staphylococci* correlates highly (p<0.001) with progesterone (Table 2). The positive effect of progesteronemia was recorded on *Staphylococcal* proliferation during the 3rd, 5th and 6th week after the part (p<0.05-0.001). The negative effect of 17 β oestradiol was recorded on the number of *Staphylococci* (p<0.01) during the 2nd week after calving (Table 2).

The numbers of *Streptococci* identified in the 10 camels during the 6 weeks after farrowing are presented in Table 3. The number of *Streptococci* was high during the first week of the postpartum, followed by a decrease of this genus until the fourth week after the part. After this period, there is a slight recovery in the kinetics of *Staphylococcal* development from the 4th to the 6th week after parturition (Table 3). There is no statistical difference in the rates of *Streptococci* between 6 weeks of study (p>0.05).

Table 4: Bacterial prevalence species in the uterine microbiota of she-camel during the 6 weeks of the post-partum

<i>Streptococci</i>	Percentage	<i>Staphylococci</i>	Percentage
<i>Lactococcus lactis</i> subsp <i>lactis</i>	13.0	<i>Staphylococcus lentus</i>	50
<i>Lactococcus lactis</i> subsp <i>cremoris</i>	8.6	<i>Staphylococcus xylosus</i>	50
<i>Leuconostoc</i> spp.	8.6		
<i>Aerococcus viridans</i> 1	8.6		
<i>Aerococcus viridans</i> 2	8.6		
<i>Aerococcus viridans</i> 3	8.6		
<i>S. dysgalactiae</i> ssp. <i>Equisimilis</i>	8.6		
<i>Streptococcus pyogenes</i>	4.6		
<i>Streptococcus pneumoniae</i>	4.4		
<i>Enterococcus faecium</i>	4.4		
<i>Streptococcus bovis</i>	4.4		
<i>Enterococcus faecalis</i>	4.4		
<i>Enterococcus avium</i>	4.4		
<i>Streptococcus uberis</i>	4.4		
<i>Streptococcus mitis</i> 1	4.4		

The number of bacteria (p<0.01) is inversely proportional to the plasma level of 17 β-estradiol (Table 3). A significant effect was observed between the number of *Streptococci* (p<0.01-0.001) and the progesterone level during the 1st, 3rd, 5th and 6th week after calving (Table 3). The negative effect of 17 β-estradiol was recorded on the number of *Streptococci* (p<0.01) during the 2nd week after farrowing (Table 3).

Staphylococcal flora exhibited similar bacterial kinetics during 6 weeks of the part; we identified only two species *S. lentus* and *S. xylosus*. In contrast, streptococcal flora was highly heterogeneous with 15 species. It appears that *Streptococcus pyogenes* and *S. pneumoniae* were identified during the first week after calving. *Lactococcus lactis* subsp *lactis* and *Lactococcus lactis* subsp *cremoris* were detected during all the period of study. *Leuconostoc* spp. and *Enterococcus faecium* were identified during the second week, *Streptococcus bovis* and *Enterococcus faecalis* were identified during the 3rd week after parturition. *Aerococcus viridans* 3 was found during the 3rd and 6th week and *Streptococcus dysgalactiae* subsp *equisimilis* was detected during the 4th and 6th week after farrowing. *Enterococcus avium* was isolated at the 4th week after the part. *Streptococcus uberis* and *Aerococcus viridans* 1 and 2 were identified during the 5th week; however, *Streptococcus mitis* 1 was detected only during the 6th week after parturition.

The rate of the different species of *Streptococci* and *Staphylococci* recorded from 10 camels during the 42 days of the post-partum is highlighted in Table 4. We identified 15 species of *Streptococci* belonging to 5 genera (Table 4) in decreasing order, a rate of 30.68% for *Streptococcus*, 30.2% for *Lactococcus*, 26.1% for *Aerococcus* and finally, 13.02% for *Enterococcus*. The different species belonging to the 5 genera as well as

their respective frequencies are highlighted in Table 4. In contrast, we identified two species of *Staphylococci* (Table 4).

Dystocia associated with human interventions are the sources of uterine infection after calving. We followed the hormonal kinetics and the bacterial flora during 6 weeks of the postpartum and the births are without human assistance.

Skidmore (2011) state that, the kinetics of progesterone and 17 β -estradiol is inversely proportional after calving similar to ours results. Plasma concentrations of these hormones are related to recovery of follicular activity, indicative of recovery of oestrogen and diminution of progesterone. Oestrogen induces hyperplasia of the glandular epithelium of the endometrium which induces hyper secretion of the genital mucus (Noakes *et al.*, 2001). This physiological evolution is in favour of a uterine involution, favourable to the resumption of ovarian activity. This testifies to a commensal bacterial microflora of the genital tract in the camel.

Our result shows a negative feedback of oestrogen on the uterine bacterial flora in camel. Indeed when estrogenemia increases the number of bacteria decreases (Table 2 and 3) similar to finding reported in cattle, sheep and pork (Wulster-Radcliffe *et al.*, 2003). The oestrogen promotes the development of *Lactobacillus* which acidifies the vaginal pH and blocks the development of pathogenic bacteria (Babu *et al.*, 2017). In contrast, there is a positive feedback of progesterone on bacterial overgrowth, the number of bacteria increases with increasing in plasma progesterone as reported in the bovine (Lewis, 2004).

This research reports the microbiota of the camel after farrowing, this is the first report on the microbiote of the camel, except for some aspects related to the uterine pathology after parturition (Ali *et al.*, 2010). The total number of bacteria isolated is high (Table 1), however, the number of the species is relatively low, only two, *S. lentus* and *S. xylosus* coagulase negative, non-pathogenic in animals and are likely to be skin contaminants (Stepanovic *et al.*, 2005). Despite the high prevalence of *S. lentus* and *S. xylosus*, we did not observe uterine disease states. It seems that these two bacterial species are not pathogenic in the dromedary and are part of the uterine microbiota after the part.

The 15 *Streptococci* species identified, on API 20 strep are saprophytic and opportunistic bacteria and appear to be agents of the uterine microbiota of the camel and are faecal and urinary contaminants. We did not record metritis or endometritis in our camels. Even in the absence of a clinical infectious of the genital tract after

part, interpretation of the microbiological results of uterine swabs remains difficult given the wide range of acidophilic bacteria that can be isolated. Some of these bacteria are part of normal genital tract flora after calving can become pathogenic under favourable conditions (Tibary and Anouassi, 1997).

Our results showed a rate of 30.68% of *Streptococcus*, this genus is a group of environmental and commensal bacteria of natural cavities of animals and humans (Krzysciak *et al.*, 2013). *S. pyogenes* was identified at the first week of the postpartum. It is a saprophyte of the skin and is responsible of dermatitis, non-invasive pharyngitis and scarlet fever in humans (Vela *et al.*, 2017). It has been implicated in camel metritis (Mshelia *et al.*, 2012), dog conjunctivitis (Sprot *et al.*, 2012). It appears to be a saprophyte of camel skin as previously mentioned in cattle (Wang *et al.*, 2013) and ewes (Vela *et al.*, 2017).

Streptococcus bovis is commensal of the digestive tract of the man and dromedary (Ghali *et al.*, 2004). It is responsible for mastitis in cattle (Poutrel and Ryniewicz, 1984) and stillbirths in pregnant women (White *et al.*, 2002). We isolated it at the 3rd week after parturition; it is not associated with an infectious state.

S. pneumoniae is human-specific bacteria, part of the normal microflora and etiological agent of respiratory infections and is rarely isolated in animals (Kim and Weiser, 1999). This bacterium was highlighted during the first week after the share with a frequency of 4.36%. *S. uberis* is an environmental species, identified in the 4th week of the part which can infect wounds and get into the mucous membranes of humans and animals. This bacterium must find a suitable medium to express its pathogenicity; like mastitis in dairy cows (Zadoks, 2007) and nosocomial infections (Domenico *et al.*, 2015).

S. dysgalactiae spp. *Equisimilis* is responsible for a variety of pathologies in humans (Rantala, 2014) and animals (Preziuso *et al.*, 2010). It was identified during the 4th and 5th week of the postpartum; it seems to induce different utero-vaginal pathological states when conditions are favourable its development. The clinical impact of this bacterium in humans and animals is still unknown because of the lack of means of identification in clinical laboratories (Rantala, 2014).

Streptococcus mitis 1 is a saprophytic of the oral cavity and human pharynx (Engen *et al.*, 2017). Although, the epidemiology and pathogenesis of this bacterium is unknown, its clinical impact in humans is variable (Shelburne *et al.*, 2014). Our result shows a rate of 4.34% during the 6th week of postpartum which is relatively low.

Enterococcus was identified from the 2nd to the 4th week, after calving with a rate of 13.02%. This genus is saprophytic of the digestive tract of animals and humans (Hammerum, 2012) and cow (Jayarao and Oliver, 1994). We have identified *E. faecium*, *E. faecalis* and *E. avium* with a rate of 4.34%. *E. faecium* is considered a contaminant of the camel's uterus (Wernery, 1991). These 3 saprophytic species are responsible for several pathologies in humans (Mirzoyev *et al.*, 2004).

Ours findings shows that 26.1% of the bacteria is *Aerococcus* with 3 species, *A. viridans* 1-3 with a rate of 8.6% each (Table 4). It is generally considered as saprophyte of the upper airways and the skin of healthy individuals (Guccione *et al.*, 2013). It has been isolated from cow's milk with subclinical mastitis (Liu *et al.*, 2015) and from the body of pigs (Guccione *et al.*, 2013).

A rate of 30.2% of identified bacteria belongs to *Lactococcus* (*L. lactis subsp lactis* and *L. lactis subsp cremoris*) and *Leuconostoc* (*Leuconostoc* spp.). They are homo-fermented lactic acid producers (Schleifer *et al.*, 1985) and play a decisive role in the acidification of the uterine pH that is unfavourable to the development of pathogenic bacteria (Linhares *et al.*, 2011). They are the main microbiological barrier to infection by genital pathogens (Redondo-Lopez *et al.*, 1990). They play a protective role through a combination of steric exclusion and production of inhibitory substances such as hydrogen peroxide and bacteriocin substances that could affect unwanted or pathogenic strains (Tomas *et al.*, 2003). The absence of these substances has been associated with a higher prevalence of bacterial infection during pregnancy in cows (Otero and Macias, 2006) and women (Hillier *et al.*, 1993).

CONCLUSION

This research represents the first report on the uterine microbiota in she-camels after calving. This flora is rich with 15 species of *Streptococcus* and 2 species of *Staphylococcus*. A rate of 30.2% of the bacteria identified are *Lactobacillus* of which 8.6% are *Lactococcus lactis subsp cremoris*, the latter are known for their antibacterial properties at different pH levels (Raja *et al.*, 2009). These bacteria, identified on the API 20 strept and API 20 staph galleries, appear to be commensals of the uterus and constitute the uterine microbiota in the female camel after parturition. The observations of the perineum and vulva associated with vaginoscopy did not reveal mucopurulent discharge. Uterine microbiota protects the host against pathogens either by directly targeting colonizing pathogens or by indirect mechanisms that support the immune system by modulating the host's cellular

responses to pathogens. And the presence of an indigenous microflora can be beneficial for maintaining an immune defence (Engen *et al.*, 2017).

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