Comparative Bacteriology of the Vagina and Uterus of Camels (*Camelus dromedaries*) in Sokoto, Nigeria

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Abstract: Infectious infertility is a major hindrance to reproductive performance in livestock. This study was carried out to compare the bacterial profile in the vagina and uterus of camel. Swabs of the vagina and uterus were collected from 45 camel cows with unknown reproductive history at slaughter, and analysed for bacteria. *Proteus* spp. was the most common bacteria isolated from the genitalia and was more prevalent in the vagina 21 (46.7 %) than the uterus 16 (35.6 %). The relative risk (RR) of uterine infection with *Proteus* spp. was significant (RR – 4.21; p - 0.0002). There were also more isolates of *Staphylococcus epidermidis* in the vagina 7 (15.6 %) than the uterus 5 (11.1 %), and the risk of uterine infection with this bacterium was significant (RR - 6.8; *p* - 0.047). The isolation rate of *Staphylococcus aureus*, *Klebsiella pneumoniae,* and *Salmonella*s spp. in the uterus were higher than in the vagina, while the rate of isolation of *Enterobacter aerogenes* was the same in the vagina and uterus. However, there was no risk of infection. *Escherichia coli*, *Edwardsiella tarda*, *Hafnia alve,* and *Shigella sonnei* were found in the uterus alone. Overall, there were more bacteria isolated from the uterus 43 (95.6%) than the vagina 39 (86.7%). The study showed that *Proteus* spp. and *S epidermidis* were the most common bacteria in the genitalia of camels capable of causing uterine infections.

Keywords: Bacteriology, Camels, Sokoto, Sokoto,Uterus, Vagina

INTRODUCTION

The camel is an even-toed ungulate that has a characteristic fatty deposit on its back known as "hump" (Kadim *et al*., 2002). There are three surviving species of the camel; the one-hump camel, commonly known as the dromedary (*Camelus dromedarius*), the Bactrian camel (*Camelus bactrianus*), and the wild Bactrian camel (*Camelus ferus*) (Burger *et al*., 2019). The dromedary is found in the Middle East, the horn of Africa, and South Asia; while the Bactrian group are an endangered species of two-hump camel found in central Asia, northwest China and Mongolia (Faye and Konuspayeva, 2012). Camel is used for transporting goods across the desert. However, there is an increasing demand for camel meat, milk, and hides particularly in areas affected by climate change and increasing desertification due to the desert's extreme ecological conditions (Chuluunbat *et al*., 2014). Also, camel urine has been used to manage illnesses in humans (Alkhamees and Alsanad, 2017). Despite all these, camels have low reproductive efficiency due to infertility, particularly from uterine infections (Kaufmann, 2005). Infectious agents are responsible for a wide range of diseases causing infertility in animals (Givens and Marley, 2018). In the female, infections of the genital tract lead to metritis and endometritis, thereby causing temporal or permanent loss of ability to procreate (Amin *et al*., 1996; Shokri *et al*., 2010). The uterus is a sterile environment that is connected to the vagina by a closed cervix, which is only opened during coitus and parturition (Wassenaar and Panigrahi, 2014). However, uterine environment's sterility is lost due to contaminations from the vagina (Amin *et al*., 1996). Information on the bacteria in the cervicovaginal environment is essential to understand the pathogenesis of uterine infections (Shokri *et al*., 2010). A wide range of bacteria and fungi have been isolated from the camel's uterus, with history of infertility (Tibary *et al*., 2006; Shokri *et al*., 2010). Therefore, it is important to determine the bacterial load in the uterus in relation to those residents in the vagina. This will assist in understanding the pathogenesis associated with infertility in camel.

MATERIALS AND METHODS

Area of Study: This study was conducted at the Sokoto Modern abattoir, a relatively new abattoir commissioned for use on the 28th of August, 2014 for slaughter of camel, cattle, sheep, and goats. The Laboratory analysis was carried out at the Central Laboratory, City Campus, Usmanu Danfodiyo University, Sokoto, Nigeria. Sokoto city is cosmopolitan and situated at an elevation of 308m. It lies between latitude 13º and 14º N and longitude 5º and 6º E, occupying an area in the dry Sahel. The State shares boundary with the Republic of Niger to the north, Kebbi State to the south, and Zamfara State to the east. Three seasons are known in Sokoto, namely: hot season (March-May), wet season (June-October), and harmattan (November-February) (Ohunakin *et al*. 2014).

Study Animals: Camels used in this study were female dromedary (*Camelus dromedarius*) presented for slaughter at the Sokoto Modern abattoir. They were purchased from different parts of Sokoto State and the neighbouring Niger Republic. Their reproductive and clinical histories were unknown at the time of sampling. Approval for this study was obtained from the management of the abattoir and consent was obtained from each owner.

Sample Collection: A total of 45 camel cows slaughtered at the abattoir were selected using convenient sampling. About 3-4 camels were sampled during each week`s visit from April to June, 2017. Within 30 minutes of slaughter, the intact genitalia was removed, clean aseptically and incised above the cervix to expose the uterus. A sterile swab stick was passed through the incision to collect samples for culture. Similarly, a swab stick was passed into the vagina to collect sample. The pair samples were labelled properly, placed on ice packs and transported within 1 hour of collection to the laboratory (about 6 km from the abattoir) for bacteriological examination.

Bacteriological Investigation:

The swab samples were inoculated into blood agar and MacConkey agar plates and incubated aerobically at 37°C for 24-48 hrs. The colonies grown from these cultures were further sub-cultured for 24-48 hrs on differential culture media; eosin-methylene blue agar, mannitol salt agar, according to standard protocols as described by Koneman *et al.* (1997). Suspected colonies were examined for colony morphology, Gram`s reaction and motility. Gram-negative rods and Gram-positive cocci were further subjected to standard biochemical tests as previously described (Koneman *et al.,*1997).

Data Analysis: Data obtained from the study were analyzed using descriptive statistics. Graphpad (2000) was used to analyse the relative risk of uterine infections. Values of $p<0.05$ were considered statistically significant.

RESULTS

The bacteria isolated from the vagina and uterus is presented in Table 1. *Proteus* spp. was the most common bacteria isolated from the genitalia with a higher isolation rate of 21 (46.7 %) in the vagina than 16 (35.6 %) in the uterus. The relative risk of uterine infection with *Proteus* spp. was significant (RR– 4.21, *p* - 0.0002). The isolation rate of *Staphylococcus epidermidis* in the vagina 7 (15.6 %) was higher than the uterus 5 (11.1 %) with a significant (RR - 6.8; *p* - 0.047) risk of uterine infection. The isolation rate of *Staphylococcus aureus* 10 (22.2%), *Klebsiella pneumoniae* 3 (6.7%) and *Salmonella* spp. 4 (8.9%) were more in the uterus than in the vagina that had 6 (13.3 %), 2 (4.4 %) and 2 (4.4 %), respectively. There was no risk of vagina infection with *S. aureus, K. pneumoniae,* and *Salmonella* spp. The rate of isolation of *Enterobacter aerogenes* was low in both vagina and uterus. *Escherichia coli*, *Edwardsiella tarda*, *Hafnia alve,* and *Shigella sonnei* were isolated from the uterus. In all, more bacteria were isolated from the uterus 43 (95.6 %), than the vagina 39 (86.7 %).

Table 1: Comparative bacteriology of the vagina and uterus of camel slaughtered at the Sokoto Modern abattoir (n = 45)

	Vagina		Uterus		RR	*p* value
	Number	Percentage (%)	Number	Percentage (%)		
Proteus spp.	21	46.7	16	35.6	4.21	0.0002*
Staphylococcus epidermidis	7	15.6	5	11.1	6.80	0.047*
Staphylococcus aureus	6	13.3	10	22.2	0.00	1.00
Klebsiella pneumoniae	2	4.4	3	6.7	0.00	1.00
Salmonella spp.	2	4.4	4	8.9	0.00	0.91
Enterobacter aerogenes	1	2.2	1	2.2	0.00	1.00
Escherichia coli	-		1	2.2		
Edwardsiella tarda	-		1	2.2		
Hafnia alvei	-		1	2.2		
Shigella sonnei	-		1	2.2		
Isolation rate	39	86.7	43	95.6		

RR – Relative risk

Values within rows with * are statistically significant ($p < 0.05$)

DISCUSSION

Isolation of bacteria from the genital tract of camel cows has been described as a significant cause of infertility (Tibary *et al*., 2006). In the present study, *Proteus* spp. was the most common bacteria isolated from the vagina and uterus. This contradicts earlier reports in camels carried out in Maiduguri, northeastern Nigeria (Mshelia *et al*., 2014), probably due to variation in exposure of these camels to urine and faeces. *Proteus* spp. is an opportunistic pathogen commonly isolated from the bladder, kidney, and intestine (Drzewiecka, 2016). However, the bacteria have been isolated from camel cows with chronic endometritis and pyometra, as well as camel bulls with orchitis (Yagoub, 2005). In the present study, the relative risk of *Proteus* spp. infecting the uterus from the vagina was significant (RR-4.21; *p*-0.0002), probably due to the contamination of the vagina from the environment and from faecal material that smears the vagina during the breeding season (Singh *et al*., 2008). The organism could then be transmitted from the genitals of contaminated males to the vagina of the female during breeding and subsequently infecting the uterus.

There were more isolates of *Staphylococcus epidermidis* in the vagina than the uterus, with a significant risk of uterine infection (RR-6.80; *p*-0.047*). *S. epidermidis* is one of the major normal flora of the skin and mucous membranes in humans such as in the vagina (Vuong and Otto, 2002; Ji *et al*., 2009). This bacterium was initially viewed as innocuous but now regarded as a pathogen that requires favourable predisposing factors to exert its virulence (Vuong and Otto, 2002). *S. epidermidis* has been isolated from women with vulvovaginitis (Gerstner *et al*., 1982), supporting its ability to cause infection. To the best of our knowledge, *S. epidermidis* has not been isolated from the genitalia of camels before, although it has been found in the uterus of goats (Rind and Shaikh, 2000) and buffalo (Jado *et al*., 2005; Gandahi *et al*., 2009). In the present study, there was a significant risk of uterine infection with *S. epidermidis,* suggesting that the camel cows sampled may have suffered dystocia before sampling. Presence of the bacterium has been associated with dystocia in buffalo (Jado *et al*., 2005). *Staphylococcus aureus* were more common in the uterus than in the vagina of the camels examined.

This is contrary to the reports of Mshelia *et al*. (2014) in Maiduguri, Nigeria. The difference may be associated with the stage of the reproductive cycle. It has been established that bacterial colonizing the vagina differs during the different stages of the estrous cycle (Amin *et al*., 1996). That may have played out in the camels examined in this study. In addition, *S. aureus* is a common bacterium isolated from the uterus of camels with endometritis or pyometra (Wernery, 1991; Yagoub, 2005; Nabih and Osman, 2012) and uninfected camel cows (Werner*yet al*., 1992: Umaru and Obudu, 1999).

Klebsiella pneumoniae and *Salmonella* spp. were less commonly isolated from the genitalia in our study. However, these bacteria were more common in the uterus than in the vagina, although the differences were not statistically significant. These organisms have been isolated from camels with endometritis (Yagoub, 2005); Nabih and Osman. 2012) and repeat breeder (Ali *et al*., 2010), suggesting their ability to cause infection in the uterus. *Klebsiella pneumoniae* was initially classified as a pathogen incapable of infecting the uterus (Sheldon *et al*., 2002), but it has been isolated from infected uterus (Umaru and Obudu, 1999; Mandefro *et al*., 2020). The isolation rate of *Enterobacter aerogenes* in the vagina was low and not different from the uterus. This is not unexpected since *E. aerogenes* is an opportunistic organism found principally in the human gastrointestinal tract and environment (Davin-Regli, 2015). *Escherichia coli*, *Edwardsiella tarda*, *Hafnia alve,* and *Shigella sonnei* were the least isolated bacteria in the uterus and was not isolated from the vagina. This is contrary to previous reports in northeastern Nigeria (Mshelia *et al*., 2014) and Ethiopia (Mandefro *et al*., 2020), where *E. coli* was the most common bacteria isolated from the uterus and vagina. It is worthy to note that the vagina was devoid of *E. coli* despite being a reservoir for this bacterium (Sáez-López *et al*., 2016). The factors responsible for this are unknown but may be attributed to the phagocytic activities of *E. coli* in the genitalia (Filias *et al*., 2011).

The rate of bacteria isolated from the uterus was more than the vagina. This is contrary to the reports of Mshelia *et al*. (2012) and Mandefro *et al.* (2020). It is generally believed that most uterine infections are acquired from the vagina due to its continuous contact with the environment (Singh *et al*., 2008). The high rate of bacteria isolation in the uterus may suggest most of the camels sampled were in their periparturient period. This period is characterized by high rates of uterine infections due to opening of the natural barriers of the cervix at parturition that may remain open for days (Sheldon *et al*., 2006). However, most bacteria invading the uterus are naturally eliminated by the reproductive tract's local immune system (Turner *et al*., 2012). Despite this, some organisms may persist due to changes in endocrine status (Singh *et al*., 2008). The high rate of bacteria isolation in the uterus may therefore be due to breach in the innate defence mechanism of the uterus. This mechanism is responsible for the elimination of bacteria invading the uterus (Beutler, 2004).

CONCLUSION

This study revealed that *Proteus* spp., *Staphylococcus epidermidis,* and *Staphylococcus aureus* were the most common bacteria isolated from the vaginal and uterine of camel cows slaughtered at the Sokoto Modern abattoir. The isolation rate of 86.7 % and 95.6 % were obtained from the vagina and uterus, respectively. These bacteria are potential pathogens capable of causing infertility in camels, thereby lowering their productivity. Further studies are required to determine the bacterial in camel bulls that might play a role in camel reproductive problems.

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