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ADENOCARCINOMA IN THE GENITAL TRACT OF INFERTILE FEMALE DROMEDARY CAMELS

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ABSTRACT

The aim of this study was to describe the clinical and ultrasonographic findings, gross and microscopic appearances and changes in the haemogram and blood chemistry of vaginal, cervical and uterine adenocarcinoma in dromedary camels. Tissue overgrowths were detected during clinical examination and ultrasonography in the vagina (*n*=9), cervix (*n*=2) and uterus (*n*=1). All females were multipara and aged between 9 and 13 years. A common history of post-mating vaginal bleeding of these females was noticed. Specimens were taken for histopathology and immunohistochemistry. Blood samples were obtained for haematology and biochemistry. The overgrown tissue masses bled easily upon palpation. By ultrasound, these tumuors were homogenous and echogenic, but sometimes with multiple hypo-echogenic cavities. Upon necropsy, metastasis was observed in the regional lymph nodes, mesentery as well as in the liver in one case. Diagnosis was confirmed by histopathological examination as vaginal, cervical and uterine adenocarcinoma. Immunohistochemically, all specimens with adenocarcinoma showed diffuse expression of epithelial membrane antigen and carcinoembryonic antigen. Compared to healthy controls (*n*=15), camels with adenocarcinoma showed significant decreases in lymphocytes, monocytes, erythrocytes, haemoglobin, haematocrit, total protein, albumin, calcium and phosphorus and increases of globulin, alkaline phosphatase and magnesium. In conclusion, this is the first report of adenocarcinoma in the genital tract of female dromedary. The vagina was the most frequent affected organ. Vaginal bleeding and anaemia were the common clinical and laboratory findings.

Key words: Adenocarcinoma, blood chemistry, female camel, genital tract

Tumuors of the female tubular genital tract in domestic animals are relatively rare, with the exclusion of bovine uterine carcinomas and vaginal fibropapillomas, bovine and canine leiomyomas and transmissible canine venereal tumuors (Bastianello, 1982; McEntee and Nielsen, 1976; Kumar *et al,* 2007; Stilwell and Peleteiro, 2010; Agnew and MacLachlan, 2016; Schlafer and Foster, 2016). Adenocarcinoma is a type of cancer that forms in mucous-secreting glands/ cells throughout the body (Agnew and MacLachlan, 2016; Schlafer and Foster, 2016).

Vaginal adenocarcinoma arises from the glandular/secretory cells in the lining of the vagina that produce some vaginal secretions (Kumar *et al,* 2007; Schlafer and Foster, 2016).

There were few reports describing invasive carcinomas including the cervix in domestic animals, but invasion from either a uterine or vaginal carcinoma could not be excluded (McEntee and Nielsen, 1976; Schlafer and Foster, 2016).

Uterine adenocarcinoma, commonly termed endometrial carcinoma, develops from cells in the endometrium. Carcinomas of the endometrium are rare neoplasms of domestic animals, however, they are more frequent in cattle, when compared to other species (Anderson and Sandison, 1969; Agnew and MacLachlan, 2016; Schlafer and Foster, 2016).

Adenocarcinoma of the female genital tract of camels has not been reported previously. Therefore, the objectives of this paper was to study the vaginal, cervical and uterine adenocarcinomas in female infertile dromedary camels.

Materials and Methods

Animals and gynaecological examination

During routine gynaecological examination of infertile female camels (n=1621) throughout two breeding seasons (September – March, 2015/2016 and 2016/2017) at the Veterinary Teaching Hospital of Qassim university of Saudi Arabia, tissue

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overgrowths were detected in the vagina, cervix and uterus of 12 animals. Age, parity and breeding history were obtained. Ultrasound examination of the genital tract was performed using Real-time, B-mode diagnostic ultrasonic equipment (Aloka SSD 500, Tokyo, Japan) attached to a 5 MHz trans-rectal transducer.

Necropsy findings

Due to poor prognosis, 5 females with vaginal masses were euthanised and postmortem examinations were performed. The reproductive tracts were immediately removed and examined morphologically. Necropsy results were corroborated with the ultrasonic images.

Histopathology and immunohistochemistry

Tissue specimens from the masses were obtained during necropsy (n=5) and from the living animals via biopsy (n=7) and were fixed in 10% buffered formalin and processed routinely and stained with hematoxylin and eosin. These were examined under light microscope. Immunolabelling was performed using the avidin–biotin– peroxidase complex (ABC) method. Sections 5 mm were cut from the paraffin blocks and mounted on positive charged adhesive glass slides ('Clipped Corner X-tra Slides', Leica Biosystems, Wetzlar, Germany). The slides were incubated at 37°C overnight for accurate adhesion of the section to the slide, deparaffinised by incubation in the oven at 56°C for 15 minutes and inserted in xylene for 30 minutes, rehydrated by transferring into graded ethanol, then washed in phosphate buffer saline (PBS) (pH 7.2) for 5 minutes. Slides were immersed in a solution of 90 ml methanol + 10 ml hydrogen peroxide (1.5%) for 30 minutes for blocking of endogenous peroxidase. Sections were then rinsed and incubated with primary antibodies EMA and CEA. The immunolabelling was performed on an automated immunostainer with appropriate positive and negative controls. DAB was used as a chromogen (3-3\ diaminobenzidine tetrahyrochloride) and Mayer's haematoxylin as a counter label.

Haematology and biochemistry

Blood samples were collected from the affected 12 camels and from 15 clinical health controls (7-12 years old), for haematology (Complete Blood Count) and biochemistry. The red blood cell counts, white blood cell counts, differential leukocytic counts including lymphocytes, monocytes and neutrophils, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration were determined by using Vet Scan HM5 (Abaxis, Union City, CA 94587, USA). Total protein, albumin, globulin, alkaline phosphatase, creatine kinase, aspartate aminotransferase, gamma glutamyl transferase, blood urea nitrogen, calcium, phosphorus and magnesium were determined in the harvested sera using Vet Scan 2 (Abaxis, California, USA).

Statistical analysis

A student t-test was used to compare between females with adenocarcinoma and the apparently healthy camels for the haematological and biochemical values. Significant values were set at $P < 0.05$. IBM-SPSS statistical program, version 24 (2016) was used for analysis.

Results and Discussion

All affected females were infertile but multipara and aged between 9 and 13 years. Difficult mating or vaginal bleeding after copulation were the attached history. On sonogram, these masses were homogenous and echogenic, sometimes with multiple hypo-echogenic cavities (Fig 1).

Vaginal exploration revealed multiple solidary circumscribed thickening (2 - 4 patches, 4 cm in diameter) palpated in the inner vaginal wall. In 2 animals, the external opening of the cervix was felt abnormally firm, enlarged, undilatable and partially or completely closed to allow the passage to the uterus. In 1 case, the uterine body, just anterior to the cervix had firm, thick and diffuse masses. The overgrown tissues of the vagina, cervix and uterus bled easily upon palpation.

On necropsy, 3 animals of vaginal tumuors showed circumferential pattern of growth, while in 2 animals these masses were multifocal (Fig 2). All tumuors were ulcerative and necrotic. On cutting, they reveal hard white nodules. In one case, metastasis was observed in the iliac lymph node, mesentery and liver. The lymph node was clearly enlarged (5 cm in diameter), the mesentery showed numerous rosary arranged nodules of about 1 cm in diameter.

Histopathologically, vaginal (n=9), cervical (n=2), and uterine (n=1) adenocarcinomas were found. Variable sized glands lined by malignant epithelial cells with mucin secretion were observed in all animals (Fig 4). Well differentiated adenocarcinomas were observed in 11 animals and a moderately differentiated in one cervical case. Immunohistochemically,

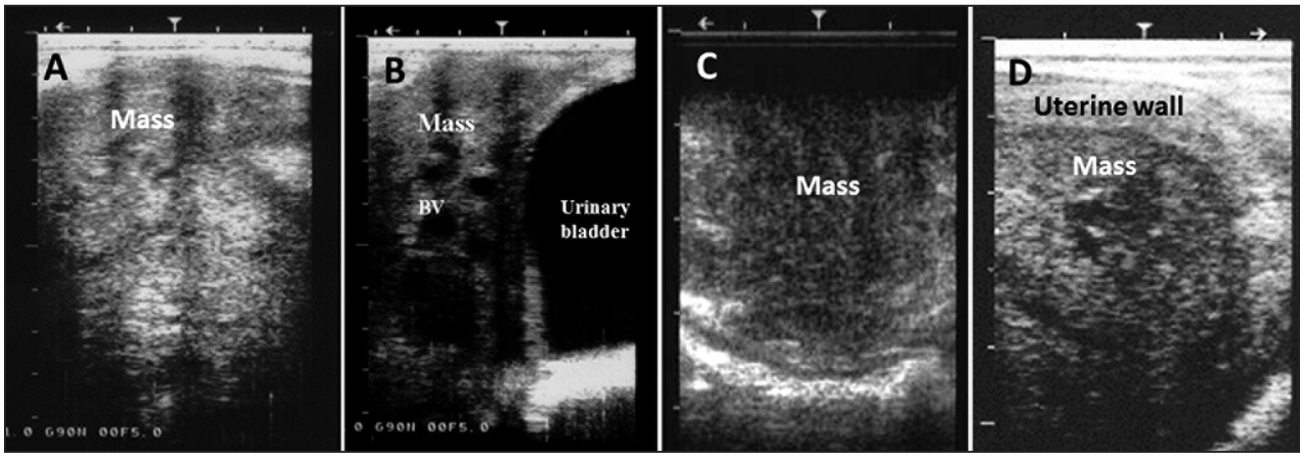

Fig 1. Sonogram of vaginal (A, B), cervical (C) and uterine (D) adenocarcinoma: the mass was echogenic, homogenous, mostly compact, but sometimes with enlarged blood vessels (BV).

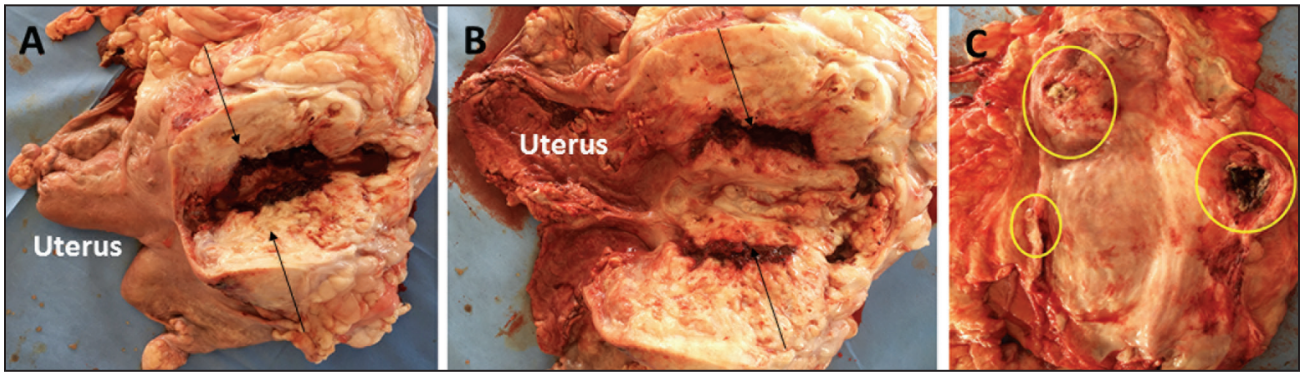

Fig 2. (A, B) Vaginal adenocarcinoma: the mass showed circumferential pattern of growth with marked narrowing of the vaginal lumen (arrows). (C) Vaginal adenocarcinoma: multifocal circumscribed ulcerated thickening in the vaginal wall.

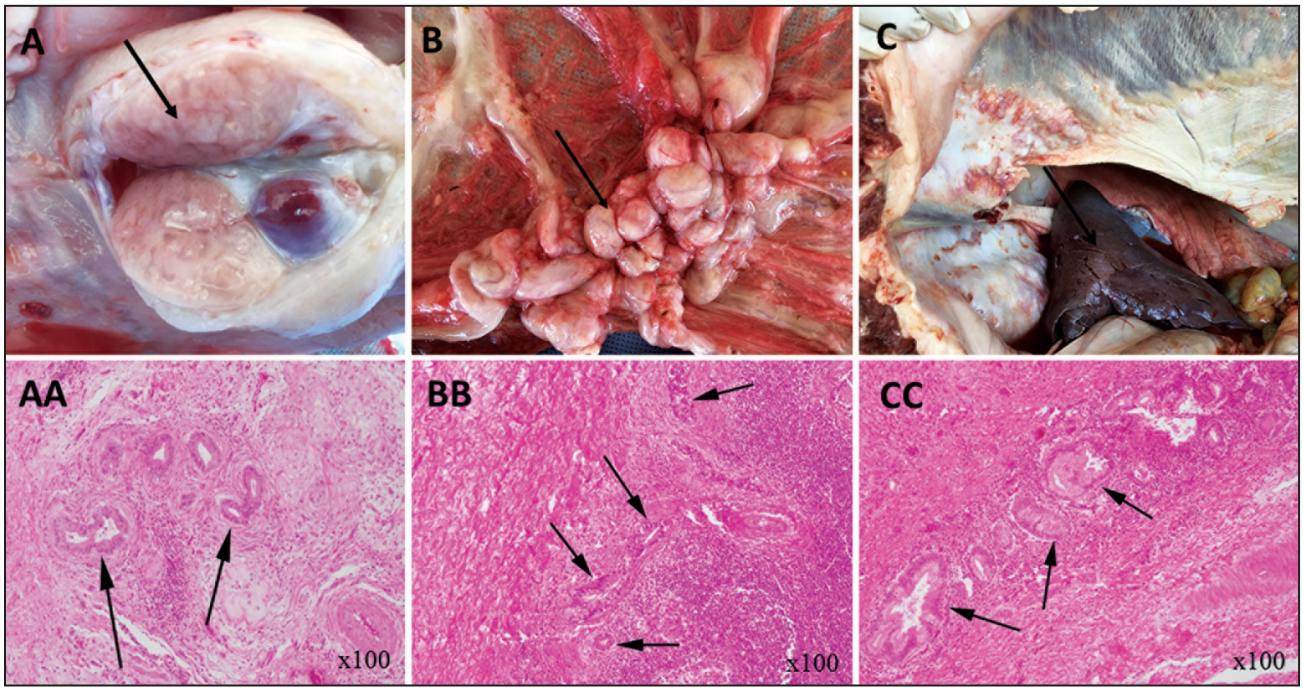

Fig 3. Metastatic vaginal adenocarcinoma. (A) The iliac lymph node was greatly enlarged (arrow). (B) Numerous small masses (1 cm in diameter) fixed to the mesentery (arrow). (C) The liver was large showed firm nodules (arrow). Multiple variable sized malignant glands (arrows) could be noticed in the mesentery (AA), lymph node (BB) and liver (CC), (H&E).

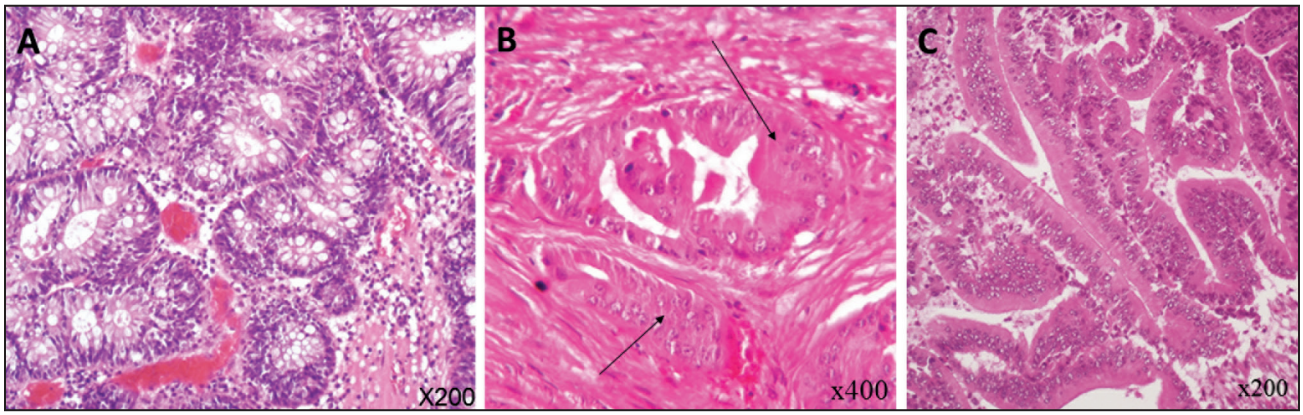

Fig 4. (A) Well differentiated invasive papillary adenocarcinoma, endometriod type, the malignant glands are lined by cuboidal tumour cells with open phase nuclei, prominent nucleoli, focal areas of haemorrhage were observed (H&E). (B) Moderately differentiated cervical adenocarcinoma (H&E). (C) Well differentiated, invasive uterine adenocarcinoma, myometrial tissue infiltrated by malignant infiltrate made up of irregularly shaped malignant glands (H&E).

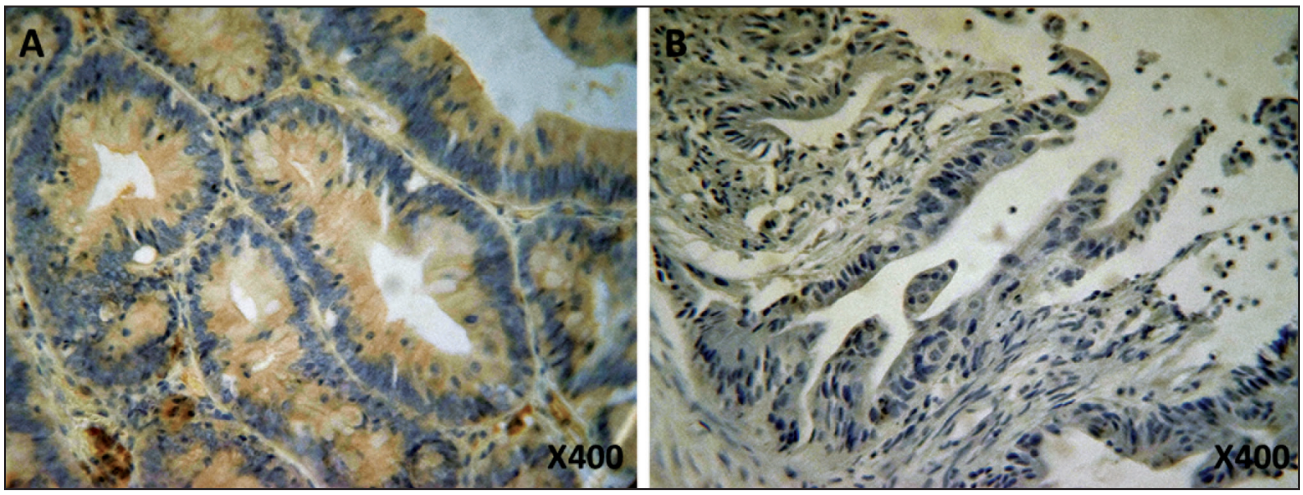

Fig 5. (A) Photomicrographs of animals with adenocarcinoma showed diffuse labelling of EMA (DAB Chromogen, H&E counter stain). (B) Photomicrographs of animals with adenocarcinoma showed labelling of CEA (DAB Chromogen, H&E counter stain).

all animals of adenocarcinoma showed diffuse cytoplasmic expression of EMA and CEA (Fig 5).

Camels with adenocarcinoma showed significant decrease in lymphocytes, monocytes, erythrocytes, haemoglobin, packed cell volume, total protein, albumin, calcium and phosphorus and increases of globulin, alkaline phosphatase and magnesium (Table 1).

The prevalence of adenocarcinoma of the genital tract of dromedary camel (0.007%) is slightly lower than that reported in cattle (0.03%) (Lucena *et al,* 2011). Clinically, affected camels showed difficult penile intromission during mating and post-mating bloody vaginal discharges were noticed. The symptoms of the tumuor differed from sero-sanguineous vaginal discharge in goat (Dockweiler *et al,* 2017), weight loss, in-appetence, mild respiratory signs, and reduced milk yield in cow (Stilwell and Peleteiro, 2010). Camels having adenocarcinoma were in middle age, however, uterine adenocarcinoma has been recorded in old cows (>6 y) (McEntee and Nielsen, 1976; Stilwell and Peleteiro, 2010).

The cause of vaginal cancer in camels is still unknown; however, papilloma virus should not be overlooked. About half of vaginal cancers in humans are associated with human papillomavirus (Viens *et al,* 2016). Papilloma viruses have been detected in camels. Non-licensed veterinary practitioners resort to place some odd materials (dates, margarine and other biological matter) inside the vagina/cervix in order to treat infertility, and these practices may additionally predispose the animal to vaginal cancers. Metastases of vaginal adenocarcinoma was detected in one case of this study. A form of contagious venereal vaginal

Table 1. Haematobiochmeical changes (mean ±SD) in camels with tumuors in compare with a control healthy group.

Blood parameter	Camels with Tumuors n=12	Control n=15	P value
White blood cell counts (10^9/L)	30.19±17.3[a]	18.85±3.58[a]	0.2
Lymphocytes (10^9/L)	1.88±0.75[a]	5.89±2.28[b]	0.003
Monocytes (10^9/L)	0.3±0.11[a]	0.89±0.55[b]	0.006
Neutrophils (10^9/L)	29.21±26.02[a]	10.79±2.89[a]	0.09
Red blood cell counts (10^{12}/L)	6.9±3.28[a]	11.38±1.31[b]	0.01
Haemoglobin concentration (g/dL)	11.48±3.34[a]	16.02±2.15[b]	0.003
Haematocrit (%)	20.5±9.27[a]	28.8±2.56[b]	0.03
Mean corpuscular volume (fl)	26±1.90[a]	25.7±1.39[a]	0.5
Mean corpuscular haemoglobin (pg)	13.40±1.07[a]	14.5±2.24[a]	0.08
mean corpuscular haemoglobin concentration (g/dL)	51.84±5[a]	57.58±8.6[a]	0.05
Albumin (G/L)	43.30±10.9[a]	60.88±2.8[b]	0.0005
Alkaline phosphatase (U/L)	55.61±23.57[a]	6.56±2.58[b]	0.001
Aspartate aminotransferase (U/L)	91.38±29.21[a]	79.5±15.5[a]	0.2
Calcium (MMOL/L)	2.1±0.16[a]	2.4±0.12[b]	0.0005
Gamma glutamyl transferase (U/L)	36.61±68.25[a]	12.18±4.98[a]	0.3
Total protein (G/L)	61.61±7.12[a]	67.25±4[b]	0.03
Globulin (G/L)	16.15±9.06[a]	6.9±3.53[b]	0.006
Blood urea nitrogen (MMOL/L)	6.36±2.37[a]	6.36±1.02[a]	0.9
Creatine kinase (U/L)	492.6±687.6[a]	138.8±20.24[a]	0.1
Phosphorus (MMOL/L)	1.55±0.32[a]	2.62±0.33[b]	0.001
Magnesium (MMOL/L)	0.82±0.16[a]	0.25±0.03[b]	0.006

[ab] Values with different superscripts in a row are significant at $P < 0.05$.

cancer has already been detected in animals (McEntee and Nielsen, 1976; Ganguly *et al,* 2016; Schlafer and Foster, 2016). This neoplasm is consisted of tumour cells creating diffuse masses or sheets underneath the mucosa. This tumour, first described 150 years ago in Europe, now has worldwide distribution (McEntee and Nielsen, 1976; Ganguly *et al,* 2016). The tumour is transmitted as intact cells by licking, by coitus, or by experimental injection. In female dogs, the neoplastic lesions are usually located at vestibule and less often at the vagina or invading the vulvar lips. Main lesions are almost always present at the junction of the vestibule and vagina. The tumour is cauliflower-like, pedunculated, nodular, papillary or multilobulated (Ganguly *et al,* 2016).

Cervical adenocarcinoma was recorded in 2 dromedary camels in this study. In fact, there were few reports describing invasive carcinomas including the cervix in domestic animals, but invasion from either a uterine or vaginal carcinoma could not be excluded (McEntee and Nielsen, 1976; Schlafer and Foster, 2016). Worldwide, cervical cancer is the fourth-most common cause of cancer and death from cancer in women (WHO, 2014). Human papillomavirus (HPV) infection appears to be involved in the development of more than 90% of cervical cancers (Kumar *et al,* 2007).

Uterine adenocarcinoma is rare in all species of domestic animal except cows and rabbits (Bastianello, 1982; Kufe, 2009; Stilwell and Peleteiro, 2010; Agnew and MacLachlan, 2016). In cows, it represents one of the 3 most common neoplasms encountered, following lymphoma and eye cancer (McEntee and Nielsen, 1976; Agnew and MacLachlan, 2016). Based on the histopathology, cancer glands invaded the myometrial layer. Similarly, the bovine form is a scirrhous adenocarcinoma that diffusely invades all layers of the wall (McEntee and Nielsen, 1976; Bastianello, 1982; Stilwell and Peleteiro, 2010). In contrast, in bitches and cats, the tumour is a non-sclerosing adenocarcinoma that typically produces a distinct mass with distortion of the mucosa (McEntee and Nielsen, 1976; Schlafer and Foster, 2016).

According to the present results, all animals of adenocarcinoma showed diffuse cytoplasmic expression of EMA and CEA. Epithelial membrane antigen (EMA) is an excellent marker of epithelial

differentiation, appears to be highly reliable for discriminating between poorly differentiated carcinomas and malignant lymphomas, and is especially helpful in characterising small cell anaplastic carcinomas (Pinkus and Kurtin, 1985). Antibodies to carcinoembryonic antigen (CEA) are commonly used in immunohistochemistry to identify cells expressing the glycoprotein in tissue samples. CEA is primarily expressed in cells of tumours but they are particularly associated with the adenocarcinomas, such as those arising in the colon, lung, breast, stomach, or pancreas. It can therefore be used to distinguish between these and other similar cancers (Ballesta *et al,* 1995).

A decrease in the number of lymphocytes and monocytes in the blood was observed in animals of present study in several diseases, but viral infections and undernutrition were the utmost common (Thrall *et al,* 2012; Roland *et al,* 2014; Vap and Bohn, 2015). The decreased erythrocytes, haemoglobin and packed cell volume may be attributed to the chronic blood loss from the haemorrhagic surface of the tumuors (Hawkey and Gulland, 1988; Stevens *et al,* 2012). The chronic long-standing nature of the tumours resulted in a significant increase in globulin but a decrease in total protein and albumin were seen (Hawkey and Gulland, 1988; Stockham and Scott, 2008; Thrall *et al,* 2012). The tumours also led to malnutrition that resulted in significant decrease of calcium and phosphorus (Garry *et al,* 1994; Tornquist, 2009; Thrall *et al,* 2012). Similar to the present study, an increase in serum alkaline phosphatase activity has been observed in human patients with ovarian cancer (Ben-Arie *et al,* 1999), metastatic breast and colon cancers (Walach and Gur, 1996; Usoro *et al,* 2010), osteosarcoma (Shimose *et al,* 2014) and skeletal metastatic cancer (Jin *et al,* 2015).

In conclusion, this is the first report pronouncing the occurrence of adenocarcinoma in the tubular genital tract of female dromedary camels. The vagina was the most frequent affected organ. Vaginal bleeding and anaemia were the commonly associated clinical and laboratory findings. Ultrasonography could be helpful in the primary diagnosis, especially in large sized masses. Most forms were diffuse and invasive. Further investigations should be focussed on associated metastasis and risk factors.

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