

ORIGINAL ARTICLE

## Pathological and bacteriological studies on reproductive tract abnormalities of she-camels (*Camelus dromedarius*), emphasizing on zoonotic importance

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### ABSTRACT

**Objective:** Infertility caused by reproductive pathologies plays a significant role in animal breeding and could result in massive economic losses to livestock owners. Hence, this study was designed to allocate various pathological lesions in the female reproductive tract of she-camels (*Camelus dromedarius*) slaughtered in Egypt and isolate the causative agents associated with those pathologies.

**Materials and Methods:** A total of 500 genitalia of adult nonpregnant she-camels aged between 6 and 15 years old were collected from three slaughterhouses at the Giza Governorate, Egypt, from August 2017 to August 2019. The uterus, cervix, and vagina were examined pathologically and microbiologically.

**Results:** The uteri of 152 cases (30.4%), cervixes of 24 cases (4.8%), and vaginae of 20 cases (4.2%) showed pathological abnormalities. The uterine inflammatory lesions were detected in 119 cases (23.8%), and the non-inflammatory lesions were detected in 58 cases (11.6%). Pathological changes of the cervix comprised 4.8%, whereas vaginal abnormalities represented 4%. The total microbial recovery rate was 28.4%, and the isolated organisms included *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, in addition to *Candida albicans*. Trials to isolate *Brucella* and *Salmonella* species were negative; however, virological examination revealed the isolation of bovine herpesvirus type-1 in two cases.

**Conclusion:** Inflammatory lesions were the most prevailing pathological lesions observed along the genital tract of she-camels, and *E. coli* was the most prevalent isolate. The microbiological burden from the genital discharge could be of zoonotic importance to the examiner and could be a contaminant to the environment and, consequently, human. In addition, attention should be paid toward the possibility of infected she-camels to transmit such infections to farm animals in contact.

### ARTICLE HISTORY

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### Introduction

Low reproductive efficiency in camelids was defined as a significant problem [1]. The camel reproductive performance under natural conditions has been widely suggested to be low [2]. Hence, reproductive disorders are essential to recognize, especially when dealing with genetically superior animals. In general, repeat breeding, refuse to mate, and difficulties in the mating process have been reported as common owner's complaints in female camels.

Uterine infections, ovulation failure, and management errors were the real reasons for camel infertility [3]. Genital defects have a significant financial influence on animal production by causing subfertility in animal breeding. Uterine disorders post-partum affect fertility by delaying uterine involution, decrease milk output, and impact the health of the animal in general [4]. As in many domestic animal species, uterine infections in she-camels were correlated with repeated breeding diseases [5]. Since the

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1980s, many prevalence surveys have been carried out in Saudi Arabia on reproductive defects [6–8], in the United Arab Emirates [9], with variable prevalence. The difference in these prevalence rates presumably concerns the application of different methods of diagnosis with variable tests and the use of different classification systems to determine abnormalities.

As compared to other animal species, there are many gaps in the understanding of the etiology and pathogenesis and evolution of genital disorders in camelids. Failure of early diagnosis and providing appropriate treatment may result in serious outcomes.

Reproductive efficiency increases are crucial for profitable production and also genetic improvement and selection of camel breeds.

Therefore, the diagnosis of reproductive disorder should occur first. Histopathological studies and isolation from the uterus are essential to identify and manage reproductive performance and circumvent reproductive disorders [10]. Camel abnormalities based on abattoir surveys of specimens could provide valuable data regarding the prevalence and incidence of reproductive disorders. In the camel, the histopathological criteria of reproductive abnormalities are largely understudied. For this reason, this study was carried out to obtain further information on the prevalence of the uterine, cervical, and vaginal lesions of she-camels slaughtered in Egypt as well as to identify their pathological problems at gross and histological levels and to isolate bacteria, fungi, and even virus from organs with abnormalities. A trial to correlate the isolated pathogens incorporated with those pathologies with probable zoonotic importance was carried out.

## Materials and Methods

### Animals

The genital tract of 500 adult non-pregnant female camels slaughtered in three abattoirs such as El-basateen, Elmonib, and Kerdasa abattoirs, Giza, Egypt, was collected and examined during the period from January 2016 to January 2018. Information about the clinical history of the animals was lacking. Reproductive organs of camel were carefully removed aseptically as possible for further examination procedures. The protocol of the current work was approved by the Institutional Animal Care and Use Committee at Cairo University, CUIIFC3118.

### Examination procedures

Gross examination was carried out on the uterus, cervix, and vagina of each case immediately following procedures of slaughtering, and any abnormalities were recorded. The specimens of each tract were numbered, includ-

ing uterus, cervix, and vagina, placed in separate sterile plastic bags, and kept frozen at  $-20^{\circ}\text{C}$ . The specimens were then brought to the laboratory for bacteriological, mycological, and virological examinations, in addition to the uterine fluid that aspirated from cases with hydrometra and pyometra, at which fluid and pus were separately collected by 5-ml sterile syringe. For histopathological and morphometrically examinations, another specimen from the previous organs was fixed in 10% buffered neutral formalin.

### Histopathological examination

Formalin-fixed tissue specimens of the previously mentioned organs were routinely processed for H&E staining. The specimens were dehydrated in a series of alcohol, followed by clearing in xylene and finally embedded in paraffin. Sections of about 4–6- $\mu\text{m}$  thickness were obtained from each paraffin block and then were routinely stained with hematoxylin and eosin (H&E) according to Bancroft and Gamble [11]. Special stains were used on need, including Massons' trichrome stain, periodic acid Schiff, Prussian blue, and Gram stain Bancroft and Gamble [11].

### Histomorphometric analysis

In some selected pathological lesions, five random high microscopic fields per tissue section were analyzed using a computer-aided microscopic image analyzer connected to a full HD microscopic camera (Leica Microsystems, Germany).

### Microbiological examination

The samples sent for microbiological examination were only for those organs which showed pathological abnormalities (196 specimens). Bacteriological examination was performed on the collected uterine, cervical, and vaginal specimens and the aspirated uterine pus and fluid samples using standard protocols [12]. Tissue samples were minced with sterile scissors and forceps. All the samples were plated onto blood agar for *Streptococcus* and *Corynebacterium* species, MacConkey agar (Oxoid), eosin methylene blue agar (Oxoid) for *Enterobacteriaceae*, Pseudomonas agar base containing (C-N) supplement (Oxoid) for *Pseudomonas* species, Rappaport-Vassiliadis medium with soya (RVS broth), xylose lysine deoxycholate agar (XLD agar) for *Salmonella* species, Baird parker agar for *Staphylococcus aureus*, dextrose tryptone agar medium for *Bacillus* species, and sabouraud dextrose agar with chloramphenicol for *Candida albicans*. The cultures were incubated at a temperature of  $37^{\circ}\text{C}$  for 24–48 h except for sabouraud dextrose agar at  $25^{\circ}\text{C}$  for 5–7 days. Suspected colonies were further examined for colony morphology, Grams characteristics, and motility, according to Brenner and Farmer [13] and Elias et al. [14]. Gram-negative

bacilli and Gram-positive cocci were subjected to standard biochemical tests, according to Quinn et al. [12]. API 20 NE, RapID™ ONE (Remel), and Staphylect Plus kit were used for further identification for *Pseudomonas* spp., Enterobacteriaceae, and *S. aureus*, respectively.

### **Molecular detection of *Brucella* species (spp) in genital organs**

DNA extraction: the extraction of DNA from genital organs was performed using the DNeasy Blood & Tissue Kit (Qiagen, Germany) according to the manufacturer's instruction. The extracted DNAs were stored at -20°C till use. Polymerase chain reaction (PCR) for the genus-specific *Brucella* cell surface salt extractable 31 kDa protein gene was performed using forward primer B4 (59 TGG CTC GGT TGC CAA TAT CAA 39) and reverse primer B5 (59 CGC GCT TGC CTT TCA GGT CTG 39) [15]. The PCR cycling conditions were 30 cycles of denaturation at 95°C for 30 sec, annealing at 54°C for 90 sec, extension at 72°C for 90 sec, and followed by a final extension at 72°C for 6 min. The PCR products were electrophoresed on 1.5% agarose to detect specific bands at 223-bp.

### **Virological examination**

Samples from the uterus, cervix, and vagina of some selected cases based on histopathological observations were pooled. It was performed, according to Khans et al. [16]. Briefly, 2–3-day-old monolayer of Madin-Darby Bovine Kidney cell line, grown in flat-bottom 24-well tissue culture plates, was injected with 200 µl of the previously prepared supernatant per well. The plates were incubated for 1 h at 37°C, and then, the inoculums were replaced with maintenance medium (Earle's Minimum Essential Medium supplemented with 2% fetal calf serum) and incubated under standard culture condition (37°C, 5% CO<sub>2</sub> and 85% humidity). The cells were checked for cytopathic effects (CPEs) using an inverted epifluorescence phase-contrast trinuclear microscope with a 20× plan chromatic lens daily for the next 5–7 days.

### **Detection of bovine herpes virus type 1 using PCR assay**

DNA extraction: from the third passage of inoculated samples on MDBK cells, DNA was extracted from 200 µl of cellular suspension using QIAamp viral nucleic acid extraction kit (Qiagen, Valencia, CA) according to manufacturer's protocol. The extracted DNA was eluted in the 60 µl of elution buffer and then stored at -20°C until used for PCR assay. PCR and agarose gel electrophoresis: the sets of primers were used to amplify separate BHV-2 genes. One set [primers 4109 (5'-GCG GCG GCG GAG TCT GGC TTT GAG-3') and 4110 (5'-TCG CTG ATG TTG TTC GGA GGG

AGG TTG A-3')] was designed to amplify a 422-bp segment of the UL29 gene, which encodes the major DNA binding protein. Reactions were run as follows: pre-denaturation at 94°C for 3 min, followed by 40 cycles at 94°C for 30 sec, 68°C for 1 minute, and 72°C for 30 sec, and a final extension at 72°C for 7 min [17].

## **Results**

Examination of 500 non-pregnant she-camels for uterine, cervical, and vaginal disorders revealed pathological lesions in 152 uteri (30.4%), 24 cervixes (4.8%), and 20 vaginae (4%). The prevalence and characterization of those pathological abnormalities are shown in Table 1.

### **Seasonal incidence of the pathological alterations**

The observed pathologies were more pronounced in the summer than the other seasons of the year, followed by autumn, whereas the least number of abnormalities was observed in spring, as shown in Figure 1A.

### **Gross, histopathological, and histomorphometric findings**

#### **Uterus**

Uterine lesions constituted 30.4% of the total collected samples; 23.8% of them were classified as inflammatory lesions, and 11.6% were considered as non-inflammatory lesions.

The inflammatory lesions were observed in 119 cases; endometritis constituted the highest prevalence (18.8%) among the inflammatory lesions, followed by metritis (3%), perimetritis (1%), endomyometritis (0.6%), and finally, pyometra (0.4%).

Endometritis was observed in 94 cases (18.8%). It was classified according to their duration and the predominant type of exudate into the following types.

Acute catarrhal endometritis was the only type of acute endometritis and was detected in 33 cases (18.8%) during histopathological examination. Grossly, the endometrium was congested and edematous enlarged. Microscopically, the affected uteri showed focal epithelial desquamation and variable degrees of mononuclear inflammatory cell infiltration, particularly subepithelial and periglandular with pyknosis of the glandular nuclei (Fig. 1a). Some cases showed an amorphous golden yellow pigment (lipofuscin) deposition among the glands. Vasculitis and perivasculitis were common; the blood vessels showed edema in their walls and focal hyalinization (Fig. 1b).

Subacute endometritis was detected only in 15 cases (3%) and characterized by heavy proprial mononuclear inflammatory cell infiltration (Fig. 1c) and mild congestion, vasculitis, and perivasculitis.

**Table 1.** Incidence of various pathological lesions observed in the uterus, cervix, and vagina of the examined she-camels.

| Pathological lesion                    | Number of lesions | Percentage from examined cases (500) | Percentage from the total affected cases (157) |
|--|-------------------|--------------------------------------|--|
| <b>Uterine lesions</b>                 |                   |                                      |  |
| Endometritis                           | 94                | 18.8%                                | 59.8%  |
| Subacute endometritis                  | 15                | 3%                                   | 9.55%  |
| Acute catarrhal endometritis           | 33                | 6.6%                                 | 21.8%  |
| Chronic granulomatous endometritis     | 9                 | 1.8%                                 | 5.7%   |
| Chronic fibrosing endometritis         | 30                | 6%                                   | 19.1%  |
| Chronic cystic endometritis            | 7                 | 1.4%                                 | 4.4%   |
| Pyometra                               | 2                 | 0.4%                                 | 1.27%  |
| Endomyometritis                        | 3                 | 0.6%                                 | 1.9%   |
| Metritis                               | 15                | 3%                                   | 9.55%  |
| Perimetritis                           | 5                 | 1%                                   | 3.1%   |
| Uterine congestion                     | 3                 | 0.6%                                 | 1.91%  |
| Brown pigmentation                     | 15                | 3%                                   | 9.55%  |
| Endometrial hyperplasia                | 7                 | 1.4%                                 | 4.45%  |
| Endometrial adenomyosis                | 30                | 6%                                   | 19.1%  |
| Hydrometra                             | 3                 | 0.6%                                 | 1.9%   |
| <b>Cervical lesions</b>                |                   |                                      |  |
| Mucopurulent cervicitis                | 7                 | 1.4%                                 | 4.45%  |
| Hemorrhagic cervicitis                 | 2                 | 0.4%                                 | 1.27%  |
| Purulohemorrhagic cervicitis           | 2                 | 0.4%                                 | 1.27%  |
| Chronic granulomatous cervicitis       | 10                | 2%                                   | 6.36%  |
| Hyperplasia of the cervical epithelium | 2                 | 0.4%                                 | 1.27%  |
| Metaplasia of the cervical epithelium  | 1                 | 0.2%                                 | 0.63%  |
| <b>Vaginal lesions</b>                 |                   |                                      |  |
| Acute vaginitis                        | 7                 | 1.4%                                 | 4.45%  |
| Chronic vaginitis                      | 5                 | 1%                                   | 3.18%  |
| Chronic granulomatous vaginitis        | 8                 | 1.6%                                 | 5.09%  |
| <b>Total</b>                           | <b>157</b>        | <b>31.4%</b>                         | <b>100</b>                                     |

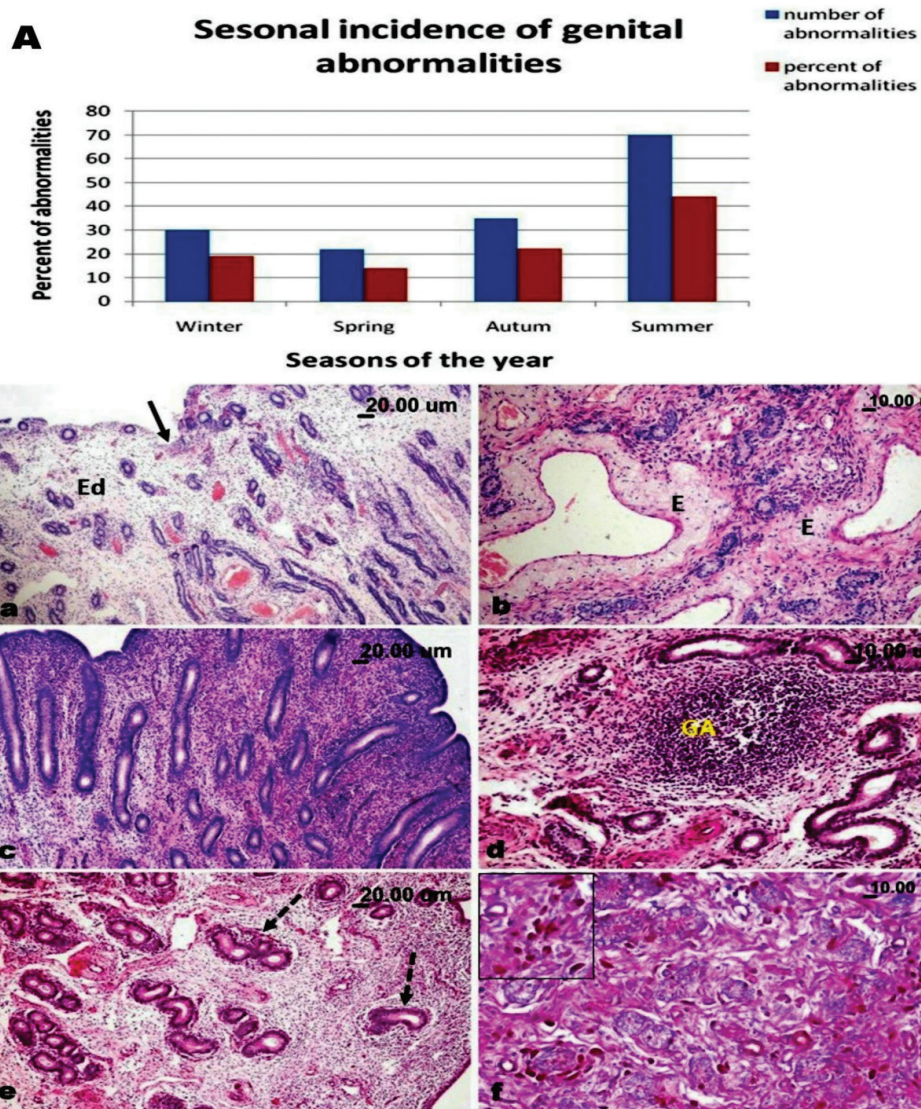
Chronic endometritis: three varieties of chronic endometritis were observed during histopathological examination such as chronic granulomatous endometritis, chronic fibrosing endometritis, and chronic cystic endometritis. Chronic granulomatous endometritis was observed in nine cases (1.8%). Apart from the pallor appearance of the uterine mucosa, no clear, gross lesions were found. However, microscopic examination revealed granulomatous aggregations of lymphocytes and macrophages subepithelially (Fig. 1d) accompanied by focal cystic or atrophic changes in the uterine glands and mild-to-moderate periglandular fibrosis.

Chronic fibrosing endometritis (chronic degenerative endometritis) was detected in 30 cases (6%). The En showed focal necrosis and desquamation of the lining epithelium with proprial fibroplasia and heavy lymphoplasmacytic infiltration. The endometrial glands appeared as islets sequestered by periglandular fibroplasia and inflammatory

cells (Fig. 1e), including mast cells, which stained positive with periodic acid Schiff stain (Fig. 1f). Some other glands appeared necrosed and showed fibrous replacement (Fig. 2a and b). Edema and hyalinization of the blood vessels' walls and perivascular fibroplasia (Fig. 2c) were common findings.

Chronic cystic endometritis was diagnosed in seven cases (1.4%) and characterized microscopically by cystic dilatation of endometrial glands, which appeared lined by flattened epithelium sometimes contained eosinophilic material in their lumens (Fig. 2d). Periglandular fibroplasia and inflammatory cell infiltration were noticed.

The morphometrical analysis of the area percentage of fibrous tissue (based on Masson's trichrome staining) in chronic fibrosing endometritis and chronic cystic endometritis (Fig. 2e) revealed 40% and 25% area percent of fibrous tissue in both types of endometritis, respectively, which were significantly higher ( $p < 0.05$ ) than in normal uteri (15%).



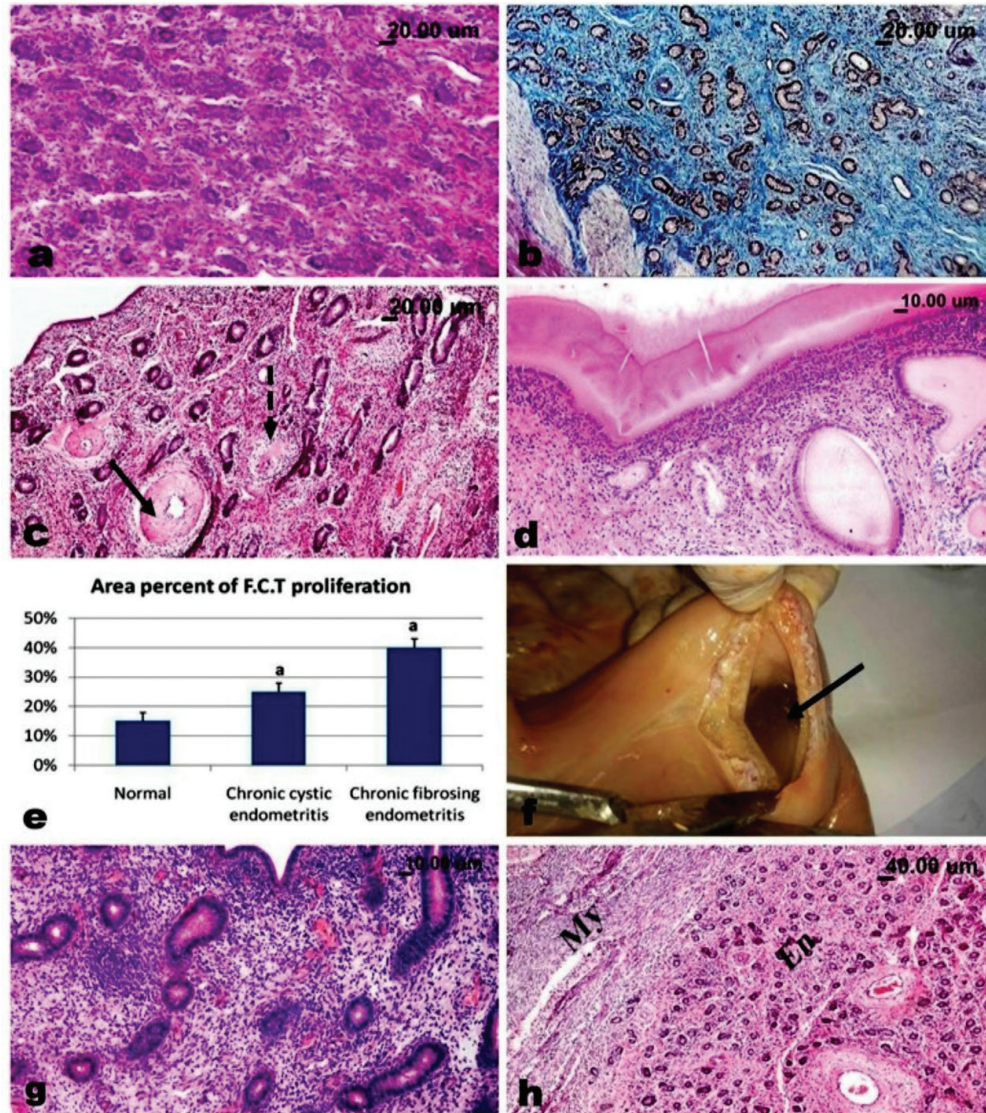
**Figure 1.** (A) Seasonal incidence of various pathological alterations in the genital tract of she-camels. (a–f) H&E stained uterine sections of she-camels. (a) Acute catarrhal endometritis revealing focal desquamation of their lining epithelium (arrow), proprial edema (Ed), mononuclear cells infiltration, and (b) edema in the blood vessels' wall (E) as well as vasculitis. (c) Subacute endometritis showing heavy mononuclear inflammatory cell infiltration. (d) Chronic granulomatous endometritis showing proprial granulomatous aggregations (GA) of lymphocytes and macrophages. (e) Chronic fibrosing endometritis showing islets (dashed arrow) of glands sequestered by periglandular fibroplasia and mononuclear inflammatory cells accompanied with (f) mast cells (positively stained with PAS) which showed marked degranulation (upper corner).

Pyometra was observed only in two cases (0.4%). The affected uteri showed a large amount of yellowish pus of foul odor in the uterine luminae (Fig. 2f) with a closed cervix. Microscopically, the mucosal epithelium was markedly necrosed and desquamated cells accompanied by heavy proprial polymorphonuclear cells infiltration, edema, and severe vasculitis (Fig. 2g). The endometrial glands showed degenerative changes and intraluminal polymorphonuclear cell infiltration. The myometrium showed an

inflammatory reaction, intermuscular edema, vasculitis, and sometimes minute foci of hemorrhages.

Endomyometritis was diagnosed in three cases (0.6%); the lesion was confined to the endometrium and myometrium without the involvement of the perimetrium. It was characterized by massive inflammatory cell infiltration (Fig. 2h).

Metritis was observed in 15 cases (3%), and it was only of acute type; the affected uteri showed only



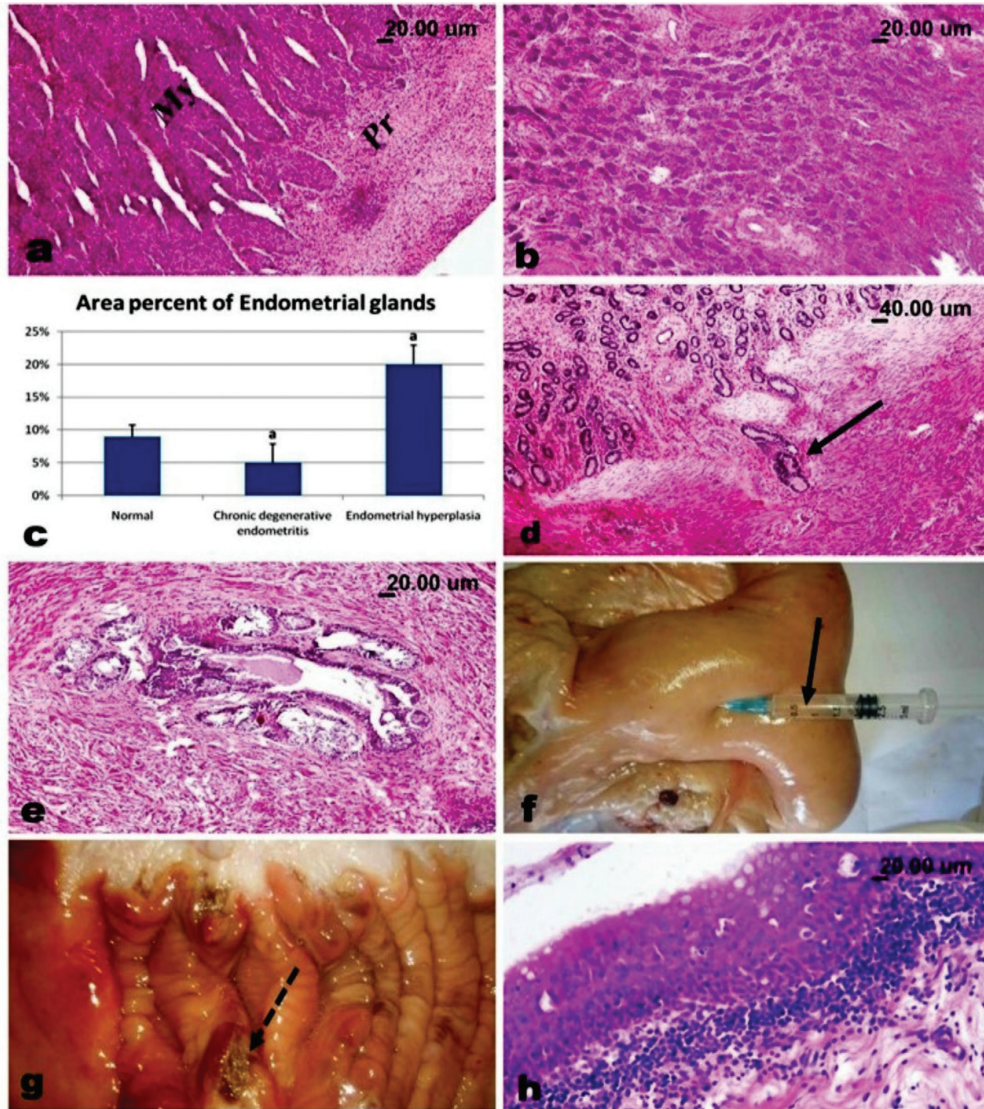
**Figure 2.** Uterine sections of she-camels. (a–c) chronic fibrosing endometritis showing: (a) degeneration, necrosis, and fibrous replacement of the endometrial glands, (b) Masson's trichrome positively stained fibroplasia, (c) necrotizing vasculitis (dashed arrow) with edema and hyalinization (arrow) of the blood vessels' walls. (d) Chronic cystic endometritis revealing cystic dilatation of some endometrial glands. (e) The area percentage of fibrous tissue in chronic fibrosing endometritis and chronic cystic endometritis (based on Masson's trichrome staining). (f and g) Pyometra; presence of large amount of yellowish pus (arrow) in the uterine lumen and (g) marked heavy polymorphonuclear cells infiltration. (h) Endomyometritis showing heavy inflammatory cell infiltration particularly periglandular in the endometrium and myometrium.

mucosal congestion. In general, it is characterized by variable degrees of focal to diffuse inflammatory cell infiltration in all uterine layers, including the Pr (Fig. 3a), accompanied by various degenerative and necrotic changes of the endometrial glands.

Perimetritis was detected microscopically in five cases (1%). It was either multifocal or diffuse and was associated with myometritis in one case. The non-inflammatory lesions were observed in 58 cases (11.6%). Adenomyosis

constituted the highest prevalence (6%), followed by brown pigmentation (3%), endometrial hyperplasia (1.4%), hydrometra (0.6%), and uterine congestion (0.6%).

Uterine congestion was observed in three cases (0.6%). Grossly, those uteri showed multifocal to coalescing or diffuse areas of congestion, accompanied by pinpoint foci of hemorrhages distributed along the endometrial mucosa and the serosal parts of the uterus. Uterine pigmentation was detected microscopically in 15 cases (3%) represented



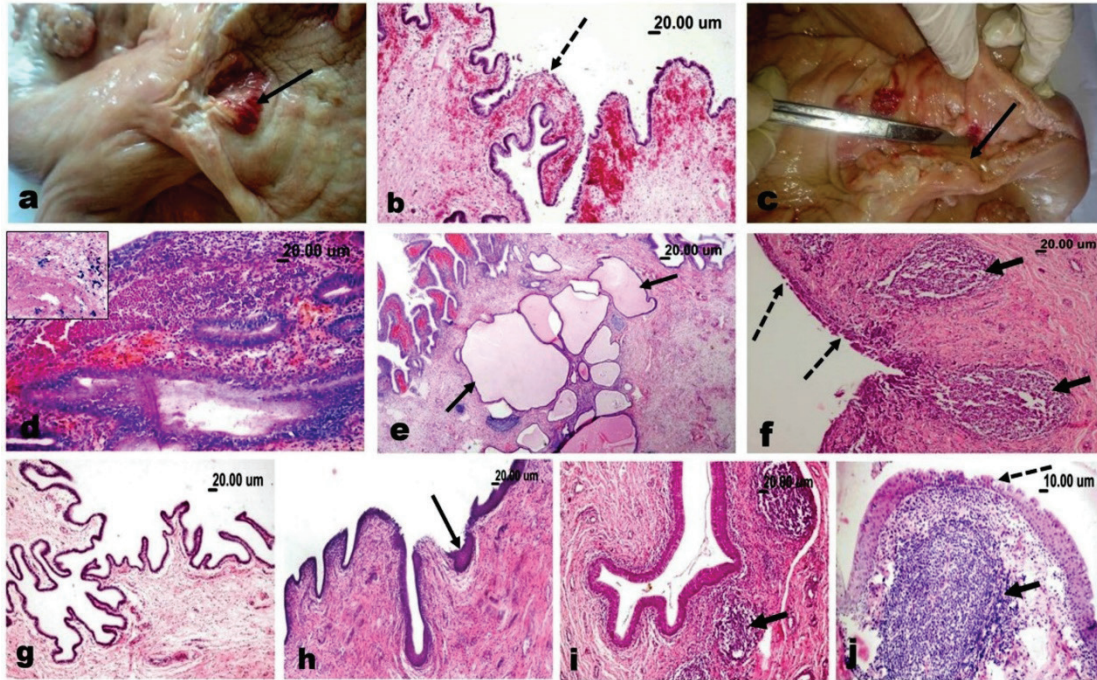
**Figure 3.** Uterine sections of she-camels. (a) Metritis; heavy inflammatory cells infiltration in the My and Pr. (b) Endometrial hyperplasia; the endometrial stroma appeared packed with an increased number of endometrial glands (gland crowding). (c) The area percentage of the endometrial glands in relation to stroma in cases of endometrial hyperplasia and in chronic fibrosing (degenerative) endometritis in relation to normal. (d and e) Uterine adenomyosis; (d) infiltrative form (arrow) and (e) presence of islet of endometrial stroma in muscular layer. (f) Hydrometra; uterine distention with a large amount of fluid (arrow). (g and h) Mucopurulent cervicitis revealing; (g) mucopurulent exudates covered the congested and ulcerated (dashed arrow) mucosa and (h) degenerative changes, hyperplasia, and goblet cells metaplasia of the mucosal epithelium with subepithelial inflammatory cell infiltration.

by the appearance of light brown pigment distributed either freely in the endometrial stroma or engulfed by large cells.

Endometrial hyperplasia was observed in seven cases (1.4%) characterized by a significantly increased number of the endometrial glands and endometrial stroma, which appeared packed with glands (gland crowding), particularly in the deeper parts (Fig. 3b). The morphometrical analysis of the area percent of the endometrial glands

about stroma showed that, in cases of endometrial hyperplasia, it was about 18%–21%, which is higher than that in the normal uteri (8%–10%). While in chronic fibrosing (degenerative) endometritis, the area percent of the endometrial glands was about 4%–5%, which is lower than that of normal uteri (Fig. 3c).

Adenomyosis was diagnosed in 30 cases (6%) with no specific gross lesions. Histologically, two forms of uterine adenomyosis were detected; the infiltrative form (Fig.



**Figure 4.** (a and b) Hemorrhagic cervicitis showing; (e) intensively red cervical mucosa with hemorrhages (arrow) and (b) necrosis and desquamation of the epithelial linings (dashed arrow), free proaprial RBCs admixed with inflammatory cell. (c and d) Purulohemorrhagic cervicitis, the mucosa covered with viscous yellowish purulent exudates (arrow) with petechial hemorrhages, (b) the propria is heavily infiltrated by neutrophils admixed with few numbers of lymphocytes, macrophages, and free RBCs and positive Prussian blue stained hemosiderin pigment granules (upper corner). (e) Multiple cervical mucosal cyst as cystic spaces (arrow) lined by flattened epithelium. (f) Chronic granulomatous cervicitis revealing multiple cellular granulomas (thick arrow) with eroded cervical epithelium (dashed arrow). (g) Hyperplasia of the cervical epithelium revealing marked folding of the cervical mucosa into variable size finger- like projections. (h) Focal cervical squamous metaplasia (arrow). (i and j) Chronic granulomatous vaginitis showing multiple granuloma (arrow) with sometimes (j) eroded (dashed arrow) mucosal epithelium over that granulomas.

3d), where the endometrial glands and stroma appeared extended in the My, is far beyond the junction between the endometrium and myometrium. The second form was characterized by islands or discrete clusters of the endometrial glands and stroma in the My with no communication with the En (Fig. 3e).

Hydrometra was observed in three cases (0.6%), and it was characterized by uterine distention with a large amount of yellow straw fluid (Fig. 3f). Microscopic examination revealed atrophy of the En, uterine glands, and My.

### Cervix

Cervical lesions were observed in 24 cases, represented 4.8% of the total collected samples. Inflammatory lesions were the most typical lesion that observed and constituted 4.2%, whereas the non-inflammatory lesions comprised only 0.6%.

Acute cervicitis was detected in eleven cases (2.2%). It was classified into mucopurulent, hemorrhagic, and purulo-hemorrhagic cervicitis.

Mucopurulent cervicitis was diagnosed in seven cases (1.4%). Grossly, the cervical mucosa was covered with

yellowish viscous pus or mucopurulent exudates with sometimes a few ulcers (Fig. 3g). Microscopically, the mucosal epithelium showed degeneration, focal desquamation, focal hyperplasia, and goblet cells metaplasia with the luminal presence of pus admixed with mucous exudates and subepithelial inflammatory cells infiltration (Fig. 3h).

Hemorrhagic cervicitis was observed in two cases (0.4%). The cervical mucosa was intensively red with variable foci of hemorrhages (Fig. 4a). Microscopical examination revealed necrosis and desquamation of the epithelial linings, vascular congestion, and proaprial extravasating blood admixed with inflammatory cell infiltration (Fig. 4b).

Purulo-hemorrhagic cervicitis was diagnosed in two cases (0.4%), in which the mucosa was covered with viscous, yellowish purulent exudates under which the mucosa was studded with petechial and larger areas of hemorrhages (Fig. 4c). Microscopically, the cervical lumen showed the presence of free RBCs admixed with a large number of neutrophils with degeneration, necrosis, and focal desquamation of the cervical epithelium and heavy proaprial infiltration by neutrophils, some



**Table 2.** The prevalence of the isolated microorganisms from different parts of she-camel genitalia and their relation to the observed histopathological lesions.

| Uterus              | Bacterial species  | No. of samples | % from total (500) | Pathological lesions revealed microbial isolation | Isolated organisms   | No. of cases | Percent from total (500) |
|---------------------|--|----------------|--------------------|---|--|--------------|--------------------------|
| A. Single infection | <i>E. coli</i>   | 31             | 6.2%               | Subacute endometritis                             | <i>E. cloacae</i> (2), <i>P. aeruginosa</i> (1), <i>E. coli</i> (3), mixed infection of <i>E. coli</i> and <i>E. cloacae</i> (7), and no growth (2)  | 15           | 3%                       |
|                     | <i>S. aureus</i>   | 7              | 1.4%               |   |  |              |                          |
|                     | <i>K. pneumoniae</i>   | 3              | 0.6%               | Acute catarrhal endometritis                      | <i>E. coli</i> (1), <i>E. cloacae</i> (1), <i>S. aureus</i> (4), mixed infection of <i>E. coli</i> and <i>E. cloacae</i> (17), mixed infection of <i>E. cloacae</i> and <i>K. pneumoniae</i> (10)  | 33           | 6.6%                     |
|                     | <i>E. cloacae</i>  | 6              | 1.2%               |   |  |              |                          |
|                     | <i>P. aeruginosa</i>   | 1              | 0.2%               |   |  |              |                          |
|                     | <i>C. albicans</i>   | 2              | 0.4%               |   |  |              |                          |
|                     |  |                |                    |   |  |              |                          |
|                     |  |                |                    | Chronic granulomatous endometritis                | <i>C. albicans</i> (1)<br><i>Staph. aureus</i> (3)<br><i>E. coli</i> (5)   | 9            | 1.2%                     |
|                     |  |                |                    | Chronic fibrosing endometritis                    | <i>E. cloacae</i> (1), <i>E. coli</i> (4), <i>K. pneumoniae</i> (2), <i>C. albicans</i> (1), Mixed infection of <i>E. coli</i> , <i>E. cloacae</i> and <i>K. pneumoniae</i> (13), mixed infection of <i>S. aureus</i> and <i>E. cloacae</i> (5), mixed infection of <i>E. coli</i> + <i>E. cloacae</i> (4) | 30           | 6%                       |
|                     |  |                |                    | Chronic cystic endometritis                       | <i>E. coli</i> (7)   | 7            | 1.4%                     |
|                     |  |                | Endomyometritis    | <i>E. coli</i> (2), No growth (1)                 | 3  | 0.6%         |                          |
| B. Mixed infection  | <i>E. coli</i> + <i>E. cloacae</i>                           | 30             | 6%                 | Pyometra  | Mixed infection of <i>S. aureus</i> , <i>Strep. pyogenes</i> and <i>P. aeruginosa</i> (2).   | 2            | 0.4%                     |
|                     | <i>E. cloacae</i> + <i>K. pneumoniae</i>                     | 10             | 2%                 | Metritis  | <i>E. coli</i> (4), <i>K. pneumoniae</i> (1), mixed infection of <i>E. coli</i> and <i>K. pneumoniae</i> (8), mixed infection of <i>E. coli</i> and <i>E. cloacae</i> (2)  | 15           | 3%                       |
|                     | <i>E. coli</i> + <i>K. pneumoniae</i>                        | 8              | 1.6%               |   |  |              |                          |
|                     | <i>E. coli</i> + <i>E. cloacae</i> + <i>K. pneumoniae</i>    | 13             | 2.6%               | Perimetritis                                      | <i>E. coli</i> (5)   | 5            | 1%                       |
|                     | <i>S. aureus</i> + <i>S. pyogenes</i> + <i>P. aeruginosa</i> | 2              | 0.4%               | Brown pigmentation                                | <i>E. coli</i> (one case which was associated with endometritis), mixed infection of <i>E. coli</i> and <i>E. cloacae</i> (6 cases, only 4 cases of them were associated with endometritis), No growth (8).  | 15           | 3%                       |
|                     | <i>S. aureus</i> + <i>E. cloacae</i>                         | 5              | 1%                 |   |  |              |                          |
|                     |  |                |                    | Endometrial adenomyosis                           | <i>E. coli</i> (10 cases which were associated with endometritis), mixed infection of <i>E. coli</i> and <i>E. cloacae</i> (10 cases which were associated with endometritis), No growth (10)  | 30           | 6%                       |
|                     | <b>Cervix</b>  |                |                    |   |  |              |                          |
| A. Single infection | <i>S. aureus</i>   | 6              | 1.2%               | Mucopurulent cervicitis                           | <i>Candida albicans</i> (2), <i>S. aureus</i> (3), mixed infection of <i>S. aureus</i> and <i>C. albicans</i> (2)  | 7            | 1.4%                     |
|                     | <i>K. pneumoniae</i>   | 2              | 0.4%               |   |  |              |                          |
|                     | <i>P. aeruginosa</i>   | 2              | 0.4%               |   |  |              |                          |
|                     | <i>C. albicans</i>   | 3              | 0.6%               | Hemorrhagic cervicitis                            | <i>P. aeruginosa</i> (2)   | 2            | 0.4%                     |
| B. Mixed infection  | <i>E. cloacae</i> + <i>K. pneumoniae</i>                     | 1              | 0.2%               | Purulo-hemorrhagic cervicitis                     | Mixed infection of <i>S. aureus</i> and <i>P. aeruginosa</i> (2)   | 2            | 0.4%                     |
|                     | <i>S. aureus</i> + <i>C. albicans</i>                        | 2              | 0.4%               | Chronic granulomatous cervicitis                  | <i>S. aureus</i> (4), <i>K. pneumoniae</i> (2), <i>C. albicans</i> (1), mixed infection of <i>S. aureus</i> and <i>K. pneumoniae</i> (1), mixed infection of <i>C. albicans</i> and <i>K. pneumoniae</i> (2)   | 10           | 2%                       |
|                     | <i>S. aureus</i> + <i>P. aeruginosa</i>                      | 2              | 0.4%               |   |  |              |                          |
|                     | <i>S. aureus</i> + <i>K. pneumoniae</i>                      | 1              | 0.2%               |   |  |              |                          |
|                     | <i>C. albicans</i> + <i>K. pneumoniae</i>                    | 1              | 0.2%               | Hyperplasia of the cervical epithelium            | -----  | 2            | 0.4%                     |
|                     | <i>S. aureus</i> + <i>C. albicans</i> + <i>K. pneumoniae</i> | 1              | 0.2%               |   |  |              |                          |

Continued

| Uterus              | Bacterial species   | No. of samples | % from total (500) | Pathological lesions revealed microbial isolation | Isolated organisms   | No. of cases | Percent from total (500) |
|---------------------|---|----------------|--------------------|---|--|--------------|--------------------------|
| Total               |   | 21             | 4.2%               | Metaplasia of the cervical epithelium             | Mixed infection of <i>E. cloacae</i> and <i>K. pneumoniae</i> (1)  | 1            | 0.2%                     |
| <b>Vagina</b>       |   |                |                    |   |  |              |                          |
| A. Single infection | <i>E. coli</i>  | 7              | 1.4%               | Acute vaginitis                                   | Mixed infection of <i>E. coli</i> and <i>E. cloacae</i> (3), <i>E. coli</i> (4)                              | 7            | 1.4%                     |
|                     | <i>S. aureus</i>  | 3              | 0.6%               |   |  |              |                          |
|                     | <i>C. albicans</i>  | 2              | 0.4%               |   |  |              |                          |
| B. Mixed infection  | <i>E. coli</i> + <i>E. cloacae</i>                        | 3              | 0.6%               | Chronic vaginitis                                 | Mixed infection of <i>E. cloacae</i> and <i>K. pneumoniae</i> (2), <i>E. coli</i> (3)                        | 5            | 1%                       |
|                     | <i>E. cloacae</i> + <i>K. pneumoniae</i>                  | 2              | 0.4%               |   |  |              |                          |
|                     | <i>E. cloacae</i> + <i>S. aureus</i> + <i>C. albicans</i> | 3              | 0.6%               |   |  |              |                          |
|                     |   |                |                    |   |  |              |                          |
| Total               |   | 20             | 4%                 | Chronic granulomatous vaginitis                   | <i>S. aureus</i> (3), <i>C. albicans</i> (2), mixed infection of <i>S. aureus</i> and <i>C. albicans</i> (3) | 8            | 1.6%                     |

lymphocytes and macrophages, free RBCs (Fig. 4d) as well as hemosiderin pigment granules which stained positive with Prussian blue. Mucosal cyst (Fig. 4e) was a conspicuous finding, appeared as subepithelial single or multiple variable sizes cystic spaces, lined by flattened epithelium with sometimes luminal homogenous eosinophilic fluid.

Chronic granulomatous cervicitis was diagnosed in 10 cases (2%). It was characterized by the presence of cellular granuloma consisted of aggregations of lymphocytes, plasma cells, and macrophages either in a focal or diffuse manner, over which the cervical epithelium was focally eroded (Fig. 4f).

The non-inflammatory lesions were limited to hyperplastic and metaplastic reactions of the cervical epithelium and detected only on microscopic examination.

The hyperplastic reaction was observed in two cases (0.4%) and characterized by marked folding of the cervical mucosa into the lumen as variable-sized finger-like projections (Fig. 4g). In comparison, focal squamous metaplasia of the cervical epithelium was detected in one case (0.2%) (Fig. 4h).

### Vagina

Vaginal lesions were observed in 20 cases, with an incidence of 4%, and were limited to an inflammatory reaction. All cases of vaginitis were associated with cervicitis, and 16 of them were associated with endometritis or metritis or perimetritis or pyometra.

Acute vaginitis was detected in seven cases (1.4%); it was characterized by vacuolation, desquamation, and focal hyperplasia of the mucosal epithelium with submucosal edema mononuclear inflammatory cell infiltration. The muscle layer revealed intermuscular edema with vacuolation and necrosis of the muscle fibers.

Chronic vaginitis was diagnosed in five cases (1%). Grossly, mucosal ulceration was observed. Microscopically, focal epithelial necrosis and desquamation, with massive lymphoplasmacytic infiltration, were characteristic findings.

Chronic granulomatous vaginitis was detected in eight cases (1.6%). Grossly, it was characterized by the thickening of vaginal walls and microscopically by the presence of multiple cellular granulomas of lymphocytes, macrophages, and plasma cells (Fig. 4i). The mucosal epithelium over those granulomas was necrotic and eroded (Fig. 4j).

### Microbiological examination

The samples which were sent for microbiological examination were only for those showed pathological abnormalities (196 specimens); it was found that 160 specimens (81.6%) were positive for bacterial isolation. As shown in Table 2, The total microbial recovery rate from she-camel's uteri was 23.6%, and the most prevalent isolated organisms were *Escherichia coli* (16.4%), *Enterobacter cloacae* (12.8%), followed by *Klebsiella pneumoniae* (4.2%) and *S. aureus* (2.8%), whereas the least prevalence was for *Pseudomonas aeruginosa* (0.6%) and *Streptococcus pyogenes* (0.4%), in addition to *C. albicans* (0.4%). The isolated organisms were represented either as single or mixed infections (Table 2). At the same time, the total microbial recovery rate from the cervix and vagina was 4.2% and 4%, respectively. The most prevalent isolated bacteria from the cervix were *S. aureus* (2.4%) followed by *K. pneumoniae* (1.2%), *P. aeruginosa* (0.8%), and finally *E. cloacae* (0.2) and *C. albicans* (1.4%) as well, whereas the most prevalent isolated bacteria from the vagina was *E. cloacae* (1.8%), followed by *E. coli* (2%), *S. aureus* (1.2%), *E. cloacae* (1%), and *K. pneumoniae* (0.4%) in addition to *C. albicans* (1%). The frequency of isolation of each organism either as a

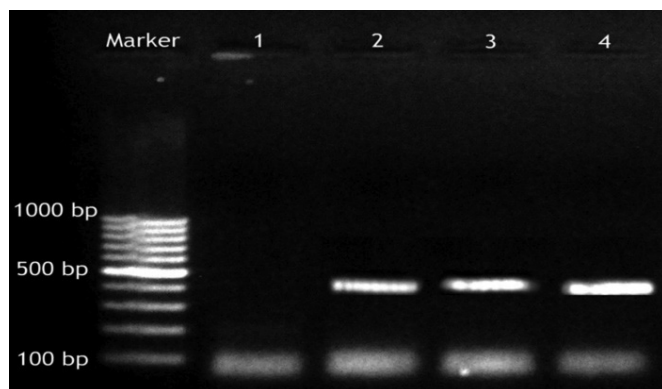
single or mixed infection with the corresponding pathological lesions is further shown in Table 2.

### Results of virological examination

On the basis of histopathological findings, five samples were subjected to the detection of the herpes virus; two samples out of five pooled samples showed positive CPE on MDBK cell line. The PCR assay for the detection of Bovine herpes virus type 1 revealed that the expected 422-bp PCR fragment was successfully amplified in 2 CPE-positive samples (Fig. 5).

### Discussion

The genital system disorders and their incidence should be allocated to reduce economic losses due to infertility problems and disorders of the genital organs, and their incidence must be allocated. In the current work, we investigated the genital of 500 she-camels that revealed pathological lesions in 152 uteri (30.4%), 24 cervixes (4.8%), and 20 vaginae (4%). Various uterine disorders have been mentioned to be incorporated in reduced fertility in camelids [6]. The detected prevalence of uterine disorders (30.4%) was higher than that recorded by Shawky et al. [18], who found that uterine affection constituted 13.2%. The uterine changes in this work were classified into inflammatory and non-inflammatory lesions. The inflammatory lesions included subacute endometritis, acute catarrhal endometritis, chronic granulomatous endometritis, chronic fibrosing endometritis, chronic cystic endometritis, endomyometritis, metritis, perimetritis, and pyometra. In general, endometritis is of significant concern that can interfere with the animal reproductive efficacy, resulting in infertility, dropped production, and economic losses [19].



**Figure 4.** PCR products of BHV-1. Lane 1, negative control; lane 2, BHV-1 positive DNA control; lanes 3 and 4 are positive samples. Amplicon size is 422-bp.

Acute catarrhal endometritis was the most common uterine inflammatory condition (6.6%), a result which was almost identical to the one cited by Shawky et al. [18], who recorded it in 6.4% in Egypt. Meanwhile, it was higher than that obtained in Southeast Algeria (2.7%) [20]. *E. coli*, *E. cloacae*, *S. aureus*, mixed infection with *E. coli* and *E. cloacae*, and mixed infection of *E. cloacae* and *K. pneumoniae* were isolated from those cases in the current results, which come in a partial agreement with Nabih and Osman [21] who isolated *S. aureus*, *E. coli*, *Corynebacterium spp.*, and *Salmonella spp.* in cases of catarrhal endometritis, whereas subacute endometritis was observed in this study in a percent of 3%, which disagrees with that mentioned by Nourani et al. [22] who diagnosed subacute endometritis in a percent of 8.3%. *E. cloacae*, *P. aeruginosa*, *E. coli*, and mixed infection of *E. coli* and *E. cloacae* were isolated from the cases of subacute endometritis. The results denoted that the incidence of granulomatous endometritis was about 1.8%.

A higher incidence (3.6%) was recorded among the imported she-camels from Sudan [23]. *Staphylococcus aureus*, *C. albicans*, and *E. coli* were isolated from those cases, a result of which was disagreed with that recorded by Hegazy et al. [23], who isolated *S. epidermidis* and *Corynebacterium pyogenes* from similar lesions. However, fungal infection was found to be a cause of granulomatous endometritis [24]. Chronic fibrosing (degenerative) endometritis was detected in 30 cases (6%). It was previously aforementioned in she-camel [3]. The degenerative changes observed during histopathological examination of these cases are mainly due to the extended periglandular and perivascular fibrosis. *E. coli*, *E. cloacae*, *K. pneumoniae*, *C. albicans*, mixed *E. coli*, *E. cloacae*, and *K. pneumoniae*, mixed infection of *E. coli* and *E. cloacae*, and mixed infection of *S. aureus* and *E. cloacae* were isolated from those cases. Regarding chronic cystic endometritis, it was observed in a percent of 1.4%, which is parallel to the results of Shawky et al. [18]. It was characterized by cystic dilatation of the endometrial glands with periglandular fibrosis. Glandular fibrosis may reduce the fertility of she-camels due to the loss of the secretory activity of such glands, which produce an unfavorable site for implantation. *E. coli* was the predominant isolate from cases with chronic cystic endometritis. In a previous study, *Micrococcus luteus*, *E. coli*, *Proteus* species, and *Citrobacter* were isolated from cases with chronic cystic catarrhal endometritis [25].

The current study revealed a low prevalence of cases with pyometra (0.4%) that is lower than that 1.1% mentioned by Benaissa et al. [20] in southeast Algeria. *Staphylococcus aureus*, *S. pyogenes*, and *P. aeruginosa* were incriminated in that condition.

Three cases (0.6%) with endomyometritis were detected in this study, from which *E. coli* was isolated. Hence, the prevalence of metritis was 3%, which is nearly similar to that reported by Benaissa et al. (2.8%) [20]. *E. coli*, *K. pneumoniae*, mixed *E. coli* and *K. pneumoniae*, and mixed *E. coli* and *E. cloacae* were isolated from the cases of metritis. On the other hand, perimetritis was detected in five cases (1%), from which *E. coli* was isolated. In camels, the penis penetrates the cervical canal deep into the uterine cavity during mating [26]. Consequently, uterine inflammatory reaction could be initiated as a result of repeated erroneous mating, which induces a sort of repeated harm to the uterus with a subsequent inability to resist infection [27]. In addition, as *Camelidae* are induced ovulators, severe uterine inflammation could be a result of mating in an aggressive way during the wrong phase of the follicular development [3,26]. Uterine resistance to infection and its self-clearance capability from microorganisms is known to be reduced in the presence of degenerative endometrial changes or heavy infection repetition with pathogenic strains [28,29]. Postpartum complications, overbreeding, and unsanitary gynecological manipulation are major factors participating in uterine infection [29]. Regarding the non-inflammatory lesions of the uterus, adenomyosis constituted the highest incidence (6%), followed by uterine pigmentation (3%), cystic endometrial hyperplasia (1.4%), and then hydrometra and uterine congestion each represented 0.6%. The observed prevalence (6%) of adenomyosis was higher than that reported in Iran (3.75%) by Wajid [30]. It was either alone or associated with an inflammatory lesion, but the prevalence of them was numerically higher in animals with endometritis, which was similarly reported in cows [31]. Adenomyosis could be an endometrial stroma hyperplastic overgrowth or malformation, and it is common in all species [32]. It is noteworthy that no bacteria were isolated from cases of adenomyosis except when associated with an inflammatory condition, which agreed with Hegazy et al. [23]. Moreover, excessive estrogenic stimulation was reported to be an inducer for endometrial hyperplasia, hydrometra, and mucometra [33]. The later excessive estrogen could originate either from an endogenous source such as granulosa cell tumors and follicular cysts or from exogenous sources such as clover pastures containing estrogenic compounds and synthetic estrogens used for remedy purposes [34].

Uterine pigmentation was identified microscopically in 15 cases and was referred to lipofuscin. It was negative for hemosiderin when stained with Perl's Prussian blue stain. Lipofuscins were commonly known as wear-and-tear pigments or age pigments [35], and most domestic ruminants were reported to have uterine lipofuscins [36]. Regarding cervicitis, it was generally associated with uterine infection, which supported the views of some authors that infec-

tious agents in the vagina move to the cervix and then to the uterine lumen and could lead to cervicitis and endometritis [37]. In the current study, 21 cases of cervicitis were observed (4.2%), which is considered a higher percentage than that observed by Shawky et al. (0.4%) [18].

The non-inflammatory lesions of the cervix were limited to hyperplastic (0.4%) and metaplastic (0.2%) changes of the cervical epithelium that was mostly accompanied by the inflammatory reaction. In light of these findings, metaplasia and hyperplasia might be attributed to chronic inflammatory reactions.

The observed vaginal changes were of low incidence represented by vaginitis (4%). This low incidence could be attributed to two factors; the first is the protective effect exerted by the vaginal mucosal stratified squamous epithelial lining, which is known to proliferate and mature under the estrogen influence and to be more resistant to infection, whereas the second is due to local production of lactic acid by normal resident *Lactobacillus* bacteria in the vagina which deposits into the epithelium [38]. In general, the cause of this vaginal affection is usually traumatic during coitus, particularly in young females [9]. Vaginitis (4%) was the only vaginal disorder observed in the current work, in which prevalence is higher than that reported by Shawky et al. (0.6%) [18]. An inflamed vagina may have resulted from inadequate hygienic conditions during parturition, postpartum, and vaginal examinations. Bacteriological isolation revealed that *E. coli*, *E. cloacae*, *K. pneumoniae*, and *S. aureus* were incriminated in cases of vaginitis, whereas *S. pyogenes* (31%), *E. coli* (24%), and *S. aureus* (20%) were the most common bacterial pathogens isolated from the vagina of camels with vaginitis in Nigeria [39].

Two cases of vaginitis were positive for Herpesvirus isolation, which agreed with the results of Darwish et al. [40]. As all *Herpesviridae*, BHV-1 remains latent in infected animals and may recur under certain stress conditions, and shedding of the virus may or may not be accompanied by clinical signs [41]. Since a normal criterion of BHV-1 infection is the viral latency, it is very important to identify the apparently healthy serologically positive animals, where this provides a reliable and useful indicator for the infection status [42]. Any animal with antibodies to the virus is considered to be a carrier and potential intermittent excretory of the virus. The only exceptions are calves that have acquired passive colostral antibodies from their dam and vaccination [45]. In general, the microbiological examination of she-camel uteri with pathological lesions revealed that the most prevalent isolated organisms were *E. coli*, *E. cloacae*, *S. aureus*, and *K. pneumoniae*, whereas the least prevalence was *P. aeruginosa*, *S. pyogenes*, and *C. albicans*. These microorganisms are known to be substantial causes of uterine, cervical, and vaginal disorders in these livestock species. Such results are of high significance since

the isolated organism has zoonotic importance and can be occupationally transmitted to human during handling with those affected she-camels.

## Conclusion

The incidence of uterine, cervical, and vaginal pathologic lesions in camels slaughtered in three abattoirs in Giza, Egypt, from January 2016 to January 2018 was 30.4%, 4.8%, and 4%, respectively. Endometritis was the major reproductive problem recorded in the examined organs, and six bacterial species were incorporated in such a problem, including *E. coli*, *E. cloacae*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *C. albicans*, with the highest prevalence for *E. coli* and *S. aureus*. The role of each reproductive problem in inducing reproductive failure in such camel species needs further investigation. Female animals should be routinely microbiologically evaluated against uterine disorders, particularly before breeding seasons. This practice, coupled with appropriate antibiotics, will enhance the reproductive efficiencies of this livestock species. Moreover, good hygienic practice during handling of the affected camel should be applied to avoid the zoonotic transmission of such pathogens. Finally, the current investigation shows that the reproductive pathology and diseases in dromedary camels are more prevalent than initially assumed. Therefore, further research should be conducted to ascertain their clinical aspects and their role as causes of reproductive defects in these animals.

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## Conflict of interest

The authors declare that they have no conflicts of interest.

## Authors' contributions

M. O. Elshazly conceived, designed the study, analyzed the data, and revised the manuscript. Sahar S Abd El-Rahman contributed to the reagents/materials/analysis tools, analyzed the data, and wrote and revised the manuscript. Dalia A Hamza contributed to the reagents/materials/analysis tools and analyzed the data. Merhan E. Ali contributed to the reagents/materials/analysis tools, collected the material, analyzed the data, and wrote the manuscript.

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