

CASE REPORT

Companion or pet animals

Reproductive tract infection caused by *Kocuria kristinae* in an entire female sugar glider (*Petaurus breviceps*)

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Abstract

The presence of a cloaca in the reproductive tract of marsupials may predispose them to ascending infections. A 7-year-old, entire, female sugar glider presented with abdominal swelling. Whole-body radiographs and abdominal ultrasound revealed a mass in the abdomen. Initial treatment included meloxicam, buprenorphine and subcutaneous fluids. Abdominal exploratory surgery revealed a severe purulent distention of the reproductive tract with multiple adhesions to the intestines, both ureters, urinary bladder and spleen. Because reproductive tissue removal was not feasible, an incision in the mass was performed, the pus aspirated and it was flushed with saline. A biopsy of the affected tissue was indicative of chronic suppurative endometritis. The microbiological culture and biochemical characterisation revealed *Kocuria kristinae*. The animal was discharged with azithromycin for 1 month and there were no complications over the ensuing 9-month period. This pathogen should be considered in the differential diagnosis of reproductive tract infections in female sugar gliders.

BACKGROUND

Sugar gliders are nocturnal arboreal marsupials with specific needs, which can be kept as companion animals at home. This case concerns a reproductive tract infection in an entire female sugar glider caused by the bacterium *Kocuria kristinae*, previously undescribed in this species. In the list of reproductive disorders in marsupials, metritis/vaginitis is absent for the sugar glider. However, the anatomical features of the reproductive tract of marsupials include the presence of a cloaca and this may predispose the animals to ascending infections. The aim of this case is to describe a pathological condition that affects sugar gliders caused by a previously unreported bacterium. The pathology resembles pyometra in dogs and cats, which usually requires a surgical approach. However, performing ovariectomy in healthy sugar gliders is a considerable challenge for anatomical reasons; the technique consists of removing the central and lateral vaginal canals and it requires the identification and preservation of the ureters, which pass between the lateral canals and the central canal

and open into the urinary bladder. If surgery is not feasible, medical management as an alternative therapy should be considered, as was the case here.

K. kristinae has been reported as a normal inhabitant of healthy bovine vagina or oral mucosa in humans, but it can be a pathogen in the reproductive tract of different species, including the sugar glider, as presented here.

CASE PRESENTATION

A 7-year-old, entire, female sugar glider (*Petaurus breviceps*) presented with a 2-day history of abdominal distension. The animal was housed in a common metal cage with a 6-year-old, entire, female companion. Their diet consisted of fruits (banana, apple, pear, persimmon), vegetables (carrot, potato, green pea) and some nuts (walnut and peanut); water was offered ad libitum in a trough. Neither of the sugar gliders had been dewormed at any point. On physical examination, the animal was bright, alert and responsive, with a bodyweight of

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76 g and a body condition score of 3/5. Five percent dehydration was detected from the delayed dorsal skin tent test. The animal had abdominal distension and showed discomfort on abdominal palpation.

INVESTIGATIONS

An imaging examination was performed under gaseous sedation with sevoflurane at 3%–4% (Sevoflo, sevoflurane 100%, Abbott) and 100% oxygen. Whole-body lateral and ventrodorsal radiographs showed loss of serosal detail and a poorly defined homogenous soft tissue opacity with an ovoid-shaped mass-like lesion in the mid/caudal abdomen, resulting in marked cranial displacement of the gas-filled gastrointestinal tract (Figure 1a,b). Ultrasonography revealed an ovoid mass 3.3 cm long and 2.5 cm wide, with fairly good margins. The mass was overall hyperechoic with ill-defined central hypoechoic regions, and colour flow Doppler interrogation showed poor vascularisation (Figure 1c). The uterus was not identifiable and the kidneys showed poor bilateral corticomedullary differentiation, suggesting chronic kidney disease. Mild pyelectasia was identified in the left kidney, consistent with increased diuresis or pyelonephritis. The owner did not give permission to perform blood analyses due to cost restraints.

The following day, an exploratory laparotomy was performed. The patient was induced with sevoflurane at 7%–8% and 100% oxygen in a mask and maintained at 3%–4%, which was monitored with a capnograph; the patient was not premedicated with injectable products in order to reduce the stressful handling before the surgery. The animal was positioned in dorsal recumbency. The heart rate was monitored with Doppler (Vet BP Doppler, Mano Medical, Warsaw, Poland), which was placed on the thorax of the animal, electrodes were fixed on the limbs for an electrocardiogram, and a pulse oximeter was placed on right forelimb. The temperature was maintained through warm water-filled gloves, which surrounded the patient. The abdomen was clipped and aseptically prepared. An infiltration of lidocaine 1 mg/kg (Lidor 20 mg/ml, Richter Pharma) and bupivacaine 1 mg/kg (Bupaq 0.3 mg/ml, Richter Pharma) was administered in the midline area; doses were calculated based on clinician experience due to the absence of pharmacokinetic/dynamic data for this species. A transparent drape was used to ensure that the patient's respiratory rate could be accurately monitored (Figure 2a). The abdomen was opened through a ventral midline incision caudally to the umbilicus and maintained in position with a self-retaining retractor (Lone Star Retractor). A large reproductive tract mass was observed. Due to the severe distension, it was difficult to recognise the different reproductive anatomical parts. Unfortunately, the removal of the reproductive tract was not possible due to the presence of multiple adhesions to the uterus/vagina, ureters, urinary bladder, spleen and the intestine loops (Figure 2b). In order to relieve the pressure towards the other organs, it was decided to remove the semi-liquid content. An incision into the ventral wall of the mass was performed to aspirate the dense yellowish content through a syringe connected to a suction system (Figure 2c). In order to manipulate the fragile tissue, the uterus/vagina was isolated with cotton swabs and sustained by two hanging sutures 4/0 polyglactin 910. A sample of the

LEARNING POINTS/TAKE-HOME MESSAGES

- Description of female marsupial reproductive tract infection is scarce.
- *Kocuria kristinae* should be considered as a differential diagnosis of reproductive tract infection in entire, female sugar gliders.
- Surgical resection of reproductive tract in marsupials can be challenging.
- Medical treatment can be adequate to manage female reproductive disorders of sugar gliders.
- *Kocuria kristinae* infection should be considered a potential sugar glider zoonosis.

mucopurulent content of the mass was submitted for bacterial culture and antimicrobial susceptibility testing (Figure 2d). A biopsy of the uterus/vagina wall was sent for histopathological examination. The uterus/vagina wall was closed with 4/0 polyglactin 910 in a simple inverted interrupted pattern (Figure 2e). The abdominal cavity was flushed with warm sterile saline. The abdominal wall was sutured in the same pattern and the skin was closed with simple horizontal mattress suture with 4/0 polyglactin 910. The animal recovered uneventfully.

For the microbiological analysis, the purulent sample was inoculated onto Columbia agar with 5% 100 sheep blood (Difco) and MacConkey agar (Oxoid) and incubated overnight at 37°C in 5% CO₂. After 24 hours, pure cultures of non-haemolytic colonies of gram-positive, catalase-positive, coagulase-negative cocci were obtained. The isolate was phenotypically identified using the Vitek 2 system (Biomérieux) as *K. kristinae* with a percent identify of 94%. Identification was confirmed by molecular analysis. DNA from the isolate was purified and a fragment of the 16S rRNA gene was amplified and sequenced. Sequences were analysed using public databases. The result of the antibiogram showed that the bacterium was susceptible to amikacin, cefotaxime, ceftazidime, gentamicin, penicillin, doxycycline, trimetho-sulfamethoxazole; intermediate to ciprofloxacin and resistant to marbofloxacin and enrofloxacin.

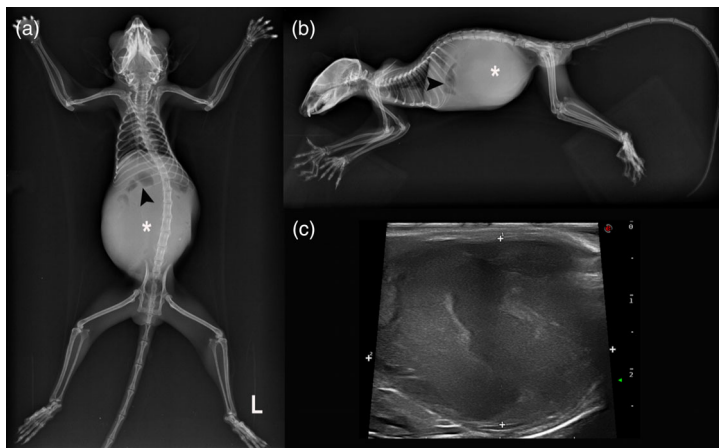
The biopsy of the uterus indicated a chronic suppurative endometritis. The uterus mucosa showed a multifocal to diffuse, mild to moderate inflammatory infiltrate consisting of lymphocytes, plasma cells and lesser amounts of scattered degenerated neutrophils. Neoplastic growth was not observed (Figure 3a). Bacteria, identified as cocci, were visualised intracellularly (Figure 3b).

DIFFERENTIAL DIAGNOSIS

The diagnostic imaging techniques revealed the presence of a mass with fluid content, with a cavitated structure being the most plausible, and almost no vascularisation.

The differential diagnoses of the abdominal mass included uterine neoplasm with necrotic component, pyometra, lymphoma, lymphadenitis, mesenteric granuloma, abscess and neoplasia.

FIGURE 1 (a and b) Whole-body lateral and ventrodorsal radiographs (L = left) showed loss of serosal details and a roundish opaque homogeneously soft tissue structure (asterisk) in the caudal abdomen. It caused a cranial displacement of the gastrointestinal tract, which contained a small amount of gas (arrowhead). (c) The abdominal ultrasonography revealed a large roundish hyperechoic structure (25.1 × 33.0 mm, between callipers) of unknown origin, with central hypoechoic regions. The ovoid structure had moderately defined margins and was slightly lateralised to the left, displacing the liver and stomach cranially and the right kidney dorsally and to the right, occupying from the middle to the caudal part of the abdominal cavity



In this case, neoplasia was less probable due to the lack of vascularisation.

At this point of the case, a uterine infection was the most probable diagnosis. During the abdominal exploratory surgery, the appearance of the distended uterus with dense fluid content was extremely helpful for the final diagnosis, which was then confirmed with the histopathological and microbiological examination.

TREATMENT

The first day, the patient was hospitalised with an initial treatment of meloxicam (0.2 mg/kg subcutaneously [SC] every 12 hours; Metacam 5 mg/ml, Boehringer), buprenorphine (0.03 mg/kg/SC/12 hours; Bupaq 0.3 mg/ml, Ritcher Pharma), warm subcutaneous fluids and assisted oral feeding with critical care formula diet for omnivores at 1 ml, three times per day (Emeraid Intensive Care Omnivore, Cornell, IL, USA).

After the surgery, the treatment plan continued as before, and adding metronidazole (20 mg/kg orally every 24 hours; Flagyl 96 25 mg/ml, Sanofi) and marbofloxacin (5 mg/kg/SC/24 hours; Marbocyl 20 mg/ml, Vetquinol), while waiting for results of the microbiological culture.

Three days later, the antibiotic treatment was changed to azithromycin (30 mg/kg/12 hours; azithromycin 40 mg/ml, Pfizer), considering the clinician's experience, the result of the bacterial culture and antimicrobial susceptibility testing. The supportive treatment continued.

OUTCOME AND FOLLOW-UP

Three weeks later, the dimension of the uterus/vagina monitored through ultrasound was 15.7 × 11.9 mm, and the medical treatment was stopped. Four months later, it was 13.9 × 15.3 mm. Eight months later, the animal is healthy and exhibiting normal behaviour.

DISCUSSION

The female reproductive tract of marsupials has a strict anatomical correlation with the urinary tract. The reproductive system consists of ovaries, oviducts and paired uterus

bodies, there are three vaginal ducts, two lateral and one median/central, which connect at the urogenital sinus, into which the urethra flows before it enters the cloaca. The presence of cloaca may predispose to ascending infections. The ovaries lie on the medioventral face of the uterus, and the convoluted oviduct is funnel-shaped and connects to the uterus, which continues until the vaginal cul-de-sac, which is divided by a septum. In sugar gliders, the median vaginal canal is short, and the lateral ones are longer. The ureters are located between the vaginal canals, which differs from the organisation in placental mammals.¹

Disorders of the reproductive tract are rarely reported in the sugar glider. Some authors have cultured *Staphylococcus aureus*, *Streptococcus* sp., *Escherichia coli* and *Proteus* sp. in female reproductive tract infections in marsupials.¹ In the short-tailed opossum (*Monodelphis domestica*), metritis and endometritis have been reported.² Cushingoid syndrome secondary to a genital tract infection was described in the Virginia opossum (*Didelphis virginiana*), and the urine culture isolated various coliforms and *Staphylococcus* sp. It was concluded that metritis leads to urinary infection; complete ovario-vaginal-hysterectomy including lateral canals was an effective cure.³

Poor husbandry conditions can affect the overall welfare status of sugar gliders. In which case, the diet is very high in carbohydrates and low in protein. It is reported that free-ranging males are likely able to meet minimal protein requirements with diets consisting of plants or insect exudates alone, while females must supplement with pollen or arthropods to meet demands of reproduction, especially during spring or summer.⁴ Because of that, dietary imbalance might lead to reproductive problems.

Leukocytosis is a common finding in reproductive tract infections. In the current case, the owner did not allow bloodwork to be performed due to the cost. Although presurgical bloodwork is normally indicated, in very small animals drawing a blood sample would drastically limit the amount of blood loss that could occur in the intraoperative period.

Medical treatment can be adequate to manage female reproductive disorders; however, sometimes ovariohysterectomy is required. This procedure can be challenging due to the position of the ureters in this species.⁵ If the ureters cannot be easily isolated, the ovaries and uterus should be removed, leaving the central and lateral vaginal canals.¹

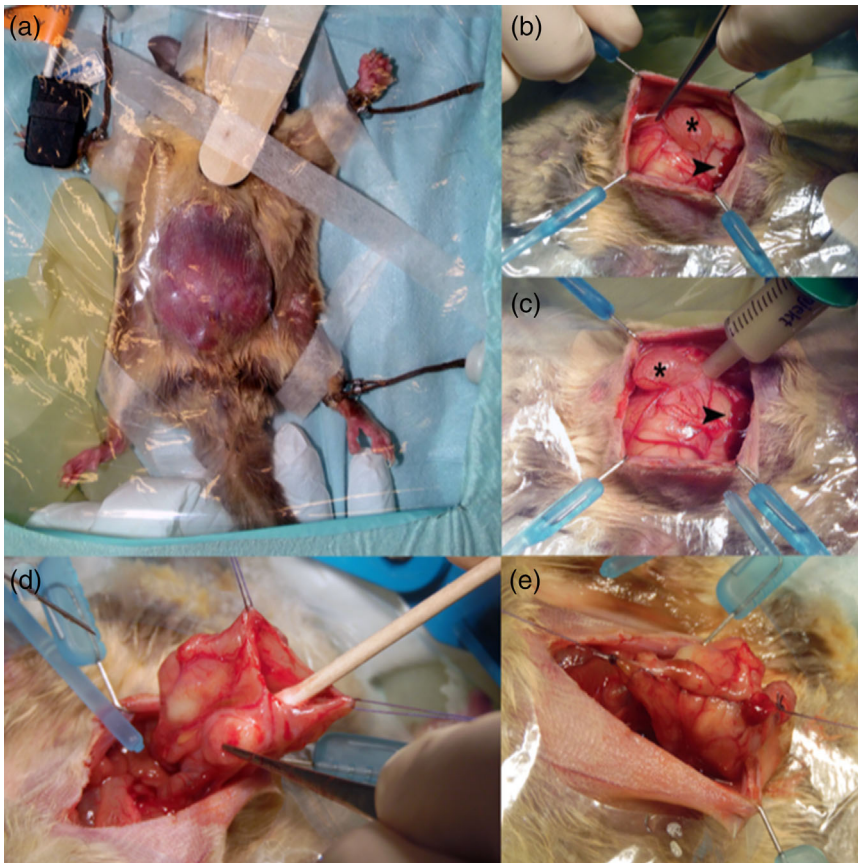


FIGURE 2 Intra-surgical photographs of a female sugar glider with a reproductive tract infection. (a) The animal was positioned in dorsal recumbency. The heart rate was monitored with Doppler (Vet BP Doppler, Mano Medical, Warsaw, Poland) placed on the thorax of the animal, electrodes were fixed on the limbs for an electrocardiogram and a pulse oximeter was placed on right forelimb. The temperature was maintained through warm water-filled gloves that enveloped the patient. The abdomen was clipped and aseptically prepared. A transparent drape was positioned to help the ease of monitoring of the patient's respiratory rate. (b) The abdomen was opened through a ventral midline incision caudally to the umbilicus and the position was maintained with the use of a self-retaining retractor (Lone Star Retractor). A large reproductive tract mass was observed, and it corresponded to the reproductive tract with adhesion to the urinary bladder (asterisk) and spleen (arrowhead). (c) An incision into the ventral wall of the mass was performed to aspirate the dense yellowish content through a syringe connected to a suction system. (d) In order to manipulate the fragile tissue, the uterus/vagina was sustained by two hanging sutures 4/0 polyglactin 910. A sample of the mucopurulent content of the mass was submitted for bacterial culture and antimicrobial susceptibility testing. (e) The uterus/vagina wall was closed with 4/0 polyglactin 910 in a simple inverted interrupted pattern

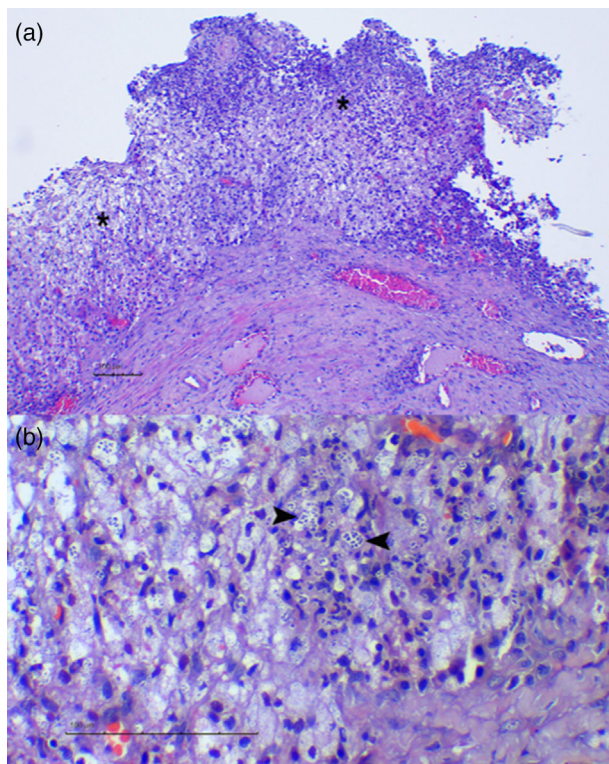


FIGURE 3 Histological images of a female sugar glider with a reproductive tract infection. (a) The histopathology of the uterus/vagina stained with haematoxylin and eosin showed the mucosa (asterisk) with a multifocal to diffuse, mild to moderate inflammatory infiltrate, consistent with lymphocytes, plasma cells and lesser amounts of scattered degenerated neutrophils. Neoplastic growth was not observed. (b) The intracellular bacteria show coccoid morphology arranged in characteristic tetrad disposition (arrowhead)

In the current case, many adhesions were visualised between the uterus, ureters, urinary bladder, spleen and small intestine. There was a loss of reproductive tract structure. The distended mass corresponded to the uterus and median and lateral vaginal canals; for this reason, it was necessary to open it and to aspirate the dense fluid content to relieve the pressure on the other organs and to avoid contamination.

In the case presented, the causative agent of the infection was identified as *K. kristinae* through biochemical characterisation.

K. kristinae belongs to the genus *Kocuria* as part of the family Micrococcaceae and class Actinobacteria. In humans, it inhabits the skin and oral mucosa, and it can cause bloodstream infection in immunocompromised individuals, but also in some instances, in immunocompetent human patients of any age.⁶ In a review, the pathogen was susceptible to vancomycin, linezolid, rifampicin, teicoplanin, tigecycline, cefotaxime, ampicillin/sulbactam, minocycline and meropenem, while resistant to penicillin, gentamycin and erythromycin.⁷ In that case, long-term medical treatment with azithromycin was successful. The antibiotic was elected as being a broad-spectrum antibiotic, it covers against aerobic and anaerobic bacteria and is available in a very oral palatable formula. The change was made considering clinician's experience, the result of the bacterial culture and antimicrobial susceptibility testing.

In veterinary literature, *K. kristinae* is not a commonly reported bacterium. *K. kristinae* has been isolated in healthy bovine vagina microbiota. It was observed to be strongly adherent to all the different types of mucus of the reproductive tract.⁸ To the authors' knowledge, this is the first report of this pathogen in marsupials, especially in sugar

gliders and it should be considered in differential diagnosis of metritis/vaginitis in this species.

AUTHOR CONTRIBUTIONS

Tiziana Bassan was the primary clinician and wrote the article. Alex Cobos performed the histopathology, informed the postmortem examination and reviewed the article. Claudia Mallol performed the transabdominal ultrasound, informed the images and reviewed the article. Maria Lourdes Abarca performed the bacterial culture and biochemical characterisation and reviewed the article. Jaume Martorell supervised the case and reviewed the article.

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CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

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OWNER'S PERSPECTIVE

The owners were very cooperative, accepting almost all the diagnostic techniques proposed. They accepted that the animal will go through the surgical procedure, understanding the related risks. During the explanation, an illustration from the book 'Quesenberry K, Carpenter JW (2020), Ferrets, Rabbits and Rodents-E-Book: Clinical Medicine and Surgery, Elsevier Health Sciences', was used in order to help them understand the difficulty of the surgery because of the arrangement of the reproductive tract very close to the urinary one. This allowed them to comprehend the difficulty of removing the tissue without damaging the ureters, due to the multiple adhesions between organs. The owners were committed to performing periodic ultrasound controls to monitor the dimension of the reproductive tract, which allowed for a good and efficient follow-up of the case.

IMAGE QUIZ

A 7-year-old, entire, female sugar glider presented with abdominal swelling. Whole-body radiographs and abdominal ultrasound revealed a mass in the abdomen. In Figure 2, you can see the exploratory laparotomy performed to treat the pathology that caused a distended abdomen.0

MULTIPLE-CHOICE QUESTION

What is the distended organ opened and what is the organ indicated by the asterisk (indicated in the part b and c of Figure 2)?

POSSIBLE ANSWERS TO MULTIPLE-CHOICE QUESTION

- Opened organ: stomach; asterisk: intestinal loop.
- Opened organ: stomach; asterisk: lymph node.
- Opened organ: uterus; asterisk: lymph node.
- Opened organ: uterus; asterisk: urinary bladder.
- Opened organ: uterus; asterisk: intestinal loop.

CORRECT ANSWER

The correct answer is number 4. The distended mass corresponded to the uterus and median and lateral vaginal canals; for this reason, it was necessary to open it and to aspirate the dense fluid content to relieve the pressure on the other organs and to avoid contamination. In the current case, many adhesions were visualised between organs: the uterus, ureters, urinary bladder (asterisk in part b and c of Figure 2), spleen (black arrow in the part c of Figure 2), and the small intestine underneath. In the case presented, the causative agent of the infection was identified as *Kocuria kristinae* through biochemical characterisation. To the authors' knowledge, this is the first report of this pathogen in marsupials, especially in sugar gliders and it should be considered in differential diagnosis of metritis/vaginitis in this species.