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## **CUTANEOUS BOTRYOMYCOSIS IN TWO PET RABBITS**

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## CUTANEOUS BOTRYOMYCOSIS IN TWO PET RABBITS

### ABSTRACT

A 5-year-old male neutered rabbit was presented for assessment of multiple abscesses, which had appeared suddenly over the ventrum, perineum and hindlimbs over the previous few days. Thirteen abscesses were surgically excised. Culture revealed *Pseudomonas aeruginosa* and histopathological examination confirmed a diagnosis of botryomycosis. However, despite follow up antimicrobial treatment and repeat surgeries, lesions continued to recur. The second case, a 3-year-old male neutered rabbit presented for routine vaccination. On physical examination, multiple small nodules were palpated over the right shoulder region. Surgical excision was performed and histopathology again yielded a diagnosis of botryomycosis. So far, no recurrence has been seen in this case. Botryomycosis is a rare chronic pyogranulomatous infection and naturally occurring disease has not been previously reported in pet rabbits. These cases highlight the challenges of treatment and the importance of prompt diagnosis.

### MANUSCRIPT

Botryomycosis is a rare chronic pyogranulomatous infection which may affect the skin, subcutis, or visceral organs.<sup>1</sup> Various bacteria may be involved and the pathogenesis is not completely understood. It is however thought that pyogranulomas develop because of a complex interaction between the virulence of the infectious organisms and the host response. This paper describes the clinical, pathological and bacteriological findings in two pet rabbits diagnosed with cutaneous botryomycosis.

A 5-year-old male neutered cross breed rabbit was referred for assessment of multiple masses, which had appeared suddenly over the last few days. The first mass had been seen following a bite wound above the tail from another rabbit eight weeks previously and treated by the referring veterinarian with oral enrofloxacin (20 mg/kg once daily, Baytril Oral Solution, Bayer, UK) for two weeks. Over the last two days however, multiple small masses had appeared, some of which were discharging white purulent material. A swab of the discharge was taken and submitted for culture and sensitivity, but results were pending at that point. On initial physical examination, 9 masses were detected over the ventrum, perineum, hind limbs and rump, ranging in size from 2-20 mm. Otherwise, clinical examination was unremarkable.

The following day, the rabbit was sedated with 0.05 mg/kg fentanyl and 1.5 mg/kg fluanisone (Hypnorm, Vetapharma Ltd, UK) by intramuscular injection. General anesthesia was induced with alfaxalone (1 mg/kg, Alfaxan, Jurox, UK) by slow intravenous injection and following intubation, maintained with 2-3% isoflurane and 2 liter/min oxygen with intermittent manual positive pressure ventilation and intravenous fluid therapy was provided at 10ml/kg/hr with compound sodium lactate (Aquapharm No. 11, Animalcare Ltd, UK) throughout the procedure. On clipping for surgical preparation, 4 further masses were detected. Surgical excision of all masses was performed except for one 3 mm mass discovered during surgery, which was left *in-situ* on the ventral surface of the prepuce. This mass was not removed due to concerns of wound dehiscence in this area. Wounds were closed with intradermal poliglecaprone 25 suture (Monocryl, Ethicon, UK) and tissue glue was applied. Each of the masses appeared to contain further encapsulated microabscesses (**Figure 1**). These were submitted for histopathological examination.

Following surgery, treatment was initiated with buprenorphine (0.05 mg/kg, Buprecare Solution for Injection, AnimalCare, UK) by subcutaneous injection and oral meloxicam (0.6 mg/kg twice daily, Loxicom Oral Suspension, Norbrook, UK) to provide analgesia, and oral ranitidine (4 mg/kg twice daily, Zantac Syrup, GlaxoSmithKline, UK) to prevent gut stasis, in addition to supplementary syringe feeding (Critical Care formula, Oxbow Animal Health, USA). Antibiotic treatment was also initiated with procaine benzylpenicillin (60,000 IU/kg once daily, Depocillin Suspension for injection, MSD Animal Health, UK) by subcutaneous injection and the rabbit remained in hospital while awaiting culture results. Over the next few days, significant urine scalding resulted in widespread dermatitis over the ventrum and wound dehiscence was seen at all surgical sites, even those on the rump. The preputial lesion had however resolved. Urinary scalding was reported by the owner at times of stress and previous investigations by the referring veterinarian had not indicated any underlying urinary problem. Frequent cleaning and topical treatment of the wounds were performed with a combination of dilute chlorhexidine (Hibiscrub, Mölnlycke Health Care Limited, UK), F10 ointment (F10® Germicidal Barrier Ointment, F10 Products Limited, South Africa) and Manuka honey (Activon, Advancis Medical, UK). Supportive treatment with analgesics, prokinetics, antibiotics and supplementary feeding were continued.

Culture results revealed a profuse growth of *Pseudomonas aeruginosa* sensitive to amikacin, gentamicin, enrofloxacin and marbofloxacin. Histopathological examination revealed that the dermis was multifocally expanded by coalescing nodules which comprised large numbers of macrophages and heterophils centered on large colonies of gram-positive cocci surrounded by brightly eosinophilic amorphous (Splendore-Hoeppli) material. In one section there was focal epidermal ulceration. The masses examined were consistent with a deep dermal bacterial infection, such as botryomycosis (**Figures 2 and 3**).

As previous treatment with enrofloxacin had not completely resolved the first mass, treatment was initiated with marbofloxacin (5 mg/kg, Marbocyl Solution for Injection, Vetoquinol UK Ltd, UK) once daily by intravenous injection in addition to the previous treatment with procaine penicillin. Wound healing was slow and metronidazole was also initiated at 20 mg/kg twice daily initially by the intravenous route (Metronidazole injection, Baxter, UK) for the first five days and then orally (Metronidazole oral suspension, Rosemont, UK). The rabbit remained in hospital for a total of 28 days on continued treatment. At this point, a further small abscess was noted under the skin in the left inguinal region and the rabbit's appetite and fecal output had reduced. Repeat surgery was considered, but the owner opted to take the rabbit home to continue on oral medications, due to concerns that the stress of hospitalization was increasing the urine scalding and hence reducing the chance of skin healing.

Three weeks later, the rabbit was re-presented for further assessment. Appetite, fecal output and urine scalding had resolved at home and all surgical wounds had now completely healed. However, on examination, the left inguinal mass noted previously had increased in size and 4 other masses had developed over the inguinal region and rump despite continued antibiotic treatment. The rabbit was therefore admitted for further investigations and surgery. A blood sample was submitted for hematology and biochemistry. Triglyceride levels were elevated at 5.24 (reference interval 1.4-1.76) mmol/L<sup>2</sup> which was consistent with the calorie dense critical care diet being fed to stimulate appetite since returning home. Occasional reactive lymphocytes were noted on examination of blood smears, but otherwise all results were

within normal limits. An abdominal ultrasound was performed to investigate the presence of internal focuses of infection, but no abnormalities were detected.

General anesthesia and surgical excision of masses were performed as previously described but again, further abscesses were detected following clipping. Samples were submitted for both tissue culture and histopathological examination. The rabbit recovered well from the procedure and was taken home the following day on continued treatment as previously described while awaiting results to reduce any stress caused by hospitalization.

Histopathology results again revealed severe chronic multifocal pyogranulomatous dermatitis and steatitis, with intra-lesional bacteria and Splendore-Hoeppli formation. However, gram staining this time revealed gram-negative intra-lesional bacteria. This was in accordance with tissue culture results which revealed a profuse growth of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. Initial sensitivity results revealed the *Pseudomonas* to now be resistant to enrofloxacin and marbofloxacin on disc diffusion tests, but minimal inhibitory concentration (MIC) testing reported the isolate to still be sensitive to these antimicrobials. Due to the recurrent nature of the disease process, euthanasia was discussed with the owners. However, the owners opted to continue treatment. Therefore, oral marbofloxacin dosage was increased to 10mg/kg once daily, while the procaine benzylpenicillin and metronidazole were discontinued.

Over the subsequent 6 months, masses continued to recur despite ongoing treatment with marbofloxacin and meloxicam. Repeat surgeries were performed to completely excise affected tissue as previously described and marsupialization techniques were also attempted to allow topical treatment with gentamicin (Tiacil Ophthalmic Solution, Virbac Limited, UK). Repeat tissue culture results again confirmed *Pseudomonas aeruginosa* infection, but with development of resistance to enrofloxacin and marbofloxacin based on MIC and disc diffusion. Potential health and safety risks were discussed with the owner, but they opted to continue palliative treatment. Nine months following initial presentation, the rabbit developed myiasis and died despite supportive care administered by the referring veterinarian. Post-mortem examination was declined by the owner.

A 3-year-old male neutered cross breed rabbit was presented for routine vaccination. No concerns were reported, but on physical examination, multiple small nodules were palpated over the right shoulder region varying from 5-8 mm in diameter. No known traumatic cause could be identified, although another rabbit was in contact, so an undetected bite wound was possible. Clinical examination was otherwise unremarkable and the rabbit was admitted for excisional biopsies of the affected region immediately. Under general anesthesia, the whole affected area was removed with ~ 15 mm surgical margins. Each of the masses contained further encapsulated microabscesses. These were submitted for tissue culture and histopathological examination. Wounds were closed with intradermal poliglecaprone 25 sutures. Histopathology revealed multilobulated and partially encapsulated abscesses, composed of numerous degenerate heterophils and macrophages, expanding the dermis and elevating the epidermis. Rarely heterophils were observed surrounding 20-80 µm diameter club-shaped colonies of 2-4 µm coccoid gram-positive bacteria surrounded by Splendore-Hoeppli material, consistent with a diagnosis of botryomycosis. Aerobic microbiology culture did not yield any bacterial growth. Recovery from anesthesia and surgery was uneventful and no recurrence was noted for 12 months.

## **Discussion**

Botryomycosis is a chronic pyogranulomatous disease, previously described in humans and a variety of companion and farm animal species including equids, cattle, dogs, hamsters, mice and guinea pigs.<sup>1, 3-8</sup> It has also been reported in a number of wild mammals including African elephants, barbary ape and harp seals.<sup>9-11</sup> Naturally occurring disease has not been previously reported in rabbits. The exact pathogenesis is not completely understood, but it is thought that pyogranulomas develop because of a complex interaction between the virulence of the infectious organisms and the host response.<sup>12</sup> The most common bacteria involved is *Staphylococcus aureus*, followed by *Pseudomonas aeruginosa*, although other bacteria such as *Proteus spp*, *Escherichia coli* and *Streptococcus spp* have been reported.<sup>1</sup> Anaerobic agents are occasionally involved, but anaerobic culture was unfortunately not performed in the cases reported herein. Disease appears to follow a traumatic injury and in humans, most patients have an altered immune response or are suffering from concurrent disease.<sup>13</sup> In the first rabbit case seen, a bite injury preceded the development of these lesions, although the relationship between the two events cannot be proven. There appeared to be a combination of infectious agents involved including gram-positive cocci identified on initial microscopic examination and, subsequently, at least one strain of *Pseudomonas aeruginosa*. The variation in sensitivity results indicate that a mixed culture is most likely in this case. In the second case, no obvious traumatic injuries had been reported by the owner, but due to the dense coat, a small puncture wound could potentially have been missed. Pathogen concentration may also be a factor. In a study in laboratory rabbits, botryomycosis did not develop when either low or high concentrations of *S. aureus* were used to induce experimental infection, but was seen when intermediate concentrations were used.<sup>14</sup>

In other species, lesions frequently affect the skin, but a visceral form also exists. This can either be a primary disease process or secondary to cutaneous infection.<sup>1</sup> In visceral disease, the lungs are frequently involved, although other organs including the liver, kidney, heart and brain may be affected.<sup>1, 15-17</sup> In these rabbit cases, there was no evidence of visceral disease, although as no post-mortem examination was performed on the first rabbit and no imaging on the second rabbit, this cannot be completely ruled out. Definitive diagnosis is based on histopathological evaluation which usually reveals characteristic heterophilic or granulomatous inflammation surrounding bacteria which are bordered by Splendore-Hoeppli material (thought to be necrotic debris admixed with antibody/antigen complexes).<sup>18</sup>

The therapy of choice for botryomycosis is surgical excision of visible lesions combined with prolonged antibiotic treatment which can extend for at least six months. Any immunosuppressive factors should also be controlled.<sup>1</sup> However, systemic antibiotic therapy may not always be effective due to failure of antibiotics to penetrate the granulomas.<sup>19</sup> Relapses appear common, as occurred in the first case and treatment is often only palliative. The potential welfare concerns of managing a case such as this should therefore be considered and discussed with owners. Euthanasia may need to be considered for severely affected or recurrent cases. The cutaneous form has been reported to be non-pruritic and non-painful in humans, but signs depend on location and size of lesions.<sup>20</sup> The first case remained on meloxicam long-term following initial diagnosis, as the location of the lesions were considered to potentially be a source of pain. The potential health and safety risks to other animals and humans of keeping a rabbit with a multiresistant bacterial infection should also be considered, although no cases of zoonotic spread from botryomycosis have been reported.

In conclusion, botryomycosis should be considered as a differential for cutaneous masses in rabbits, in particular recurrent atypical abscesses. Tissue culture and histopathological

examination is recommended for these cases and if diagnosed, prompt surgical treatment is likely to be necessary for the best outcome.

## **FIGURE LEGENDS**

Figure 1 – Initial surgical excision of masses

Figure 2 – Haired skin, rabbit. The dermis and subcutis are expanded and replaced by multiple nodules of pyogranulomatous inflammation. H&E (x20)

Figure 3 – Haired skin, rabbit. Large colony of coccoid bacteria surrounded by degenerate heterophils admixed with Splendore-Hoeppli material. \_Gram stain (x 400)

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