Extensive AMR infection with bone and bone marrow involvement in amputated tail

Cocker Spaniel Female 7 years old

Day 0" equals the first day of MPPT application

A 7-year-old cocker spaniel had what seemed like a not very severe injury to her tail. However, over the following month, the wound failed to heal and, instead, an infection developed and became increasingly evident. The dog was then prescribed systemic antibiotics amoxicillin and clavulanic acid 4:1, Synulox, 250ml 3/4 tablet twice a day, for a week and when that did not clear the infection, the same antibiotics were prescribed for a second week. As the antibiotics continued not to clear the infection, it was clear that the infection was resistant to the antibiotics, and the tail was amputated (pic 1).

Already 24 hours after the amputation, it was evident that the amputation had failed to remove the forefront of the infection which was quickly advancing and infiltrating cranially (pic 2). The infection continued to advance and grow in severity over the following days (pic 3, 4 and 5). As the pain seemed excruciating and, as the dog had become allergic to Metacam, a couple of days later the dog was prescribed Paracetamol and Gabapentin 100mg twice a day. The stitches were buried under a very thick eschar which was leaking fluid (pic 6). The vet also advised not to wash the tail and to avoid it getting wet. Furthermore, the same antibiotic to which the infection had proved resistant before the amputation, Synulox, was prescribed again (pic 5 and 6).

The antibiotics caused a change in the clinical presentation of the infection. The necrotic, infectious debris changed to a higher viscosity which made it more difficult for the immune system to expel (pic 7, 10 and 11). It showed a tendency to be retained in the soft tissue (pic 8, 9 and 12) where it could cause further harm. The necrotizing, oozing areas on the skin along the tail also turned more viscous and drier with the debris remaining on the skin as scales of eschar (pic 9), or, in more affected areas, as a top layer that can be shed (pic 8). Similarly, the slight but recognisable reduction of the swelling and redness (pic 7 and 8) indicated the shift in the nature of the infection. As these reductions were not accompanied by any signs of granulation or other type of tissue improvement, they did not indicate a reduction in the infection. This is consistent with the fact that antimicrobials induce a change in the microbiota without solving the dysbiosis that is causing the infection.

After a week, the 1 cm thick necrotic cap of eschar at the end of the stump fell off (pic 10 and 11). This revealed, a strongly necrotising infection where all the stitches had disappeared and the consumption of the soft tissue had left approximately 5mm of the severed tail bone also in an state clearly affected by the infection (pic 12). The tail was unmistakably very painful despite the continuing pain medication. The dog was withdrawn, antisocial and antagonistic towards the family's other dog as well as a human family member. This was out of character. The vet prescribed the continuation of the antibiotics, Synulox, and advised to wash the wound and apply Manuka honey. The vet also recommended a second amputation. However, the stump was short from the first amputation, and the infection was possibly already infiltrating cranially beyond the removeable area. A second amputation was therefore unlikely to remove this antibiotic resistant infection in its entirety, in which case it would continue advancing in the trunk.

2 days later, MPPT treatment was initiated (pic 13 and 14). The advancement of the necrotizing infection was stopped. Also, the soft tissue immediately started granulating and the wound edges epithelializing (pic 15 and 18). A week after starting the MPPT, the dog showed no more signs of being in pain. She was sociable, wanted to play and was active and investigative on her daily walks. Pain medication had been discontinued at the start of MPPT and a week after start the systemic antibiotics were stopped, as well. Their discontinuation benefitted and accelerated the infection removal processes.

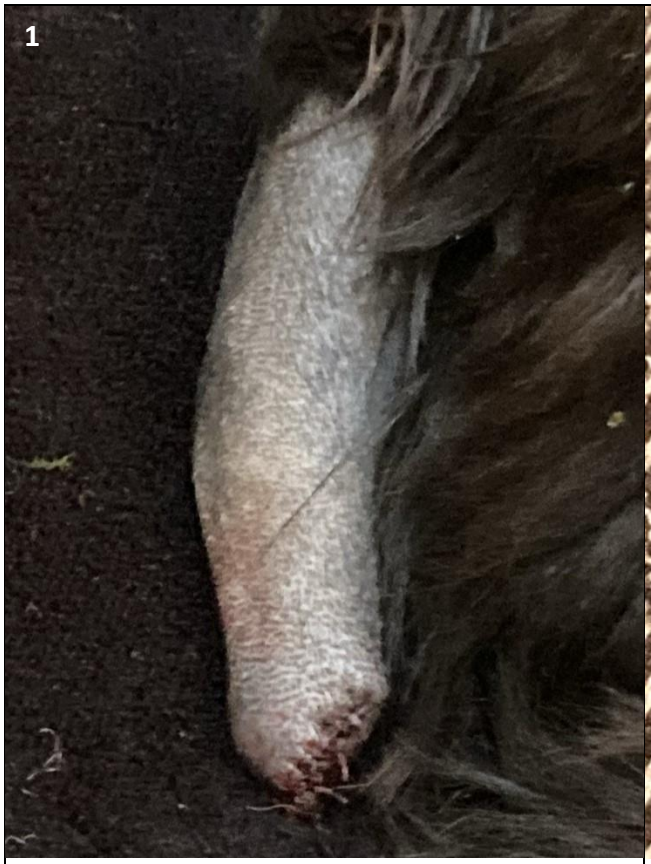
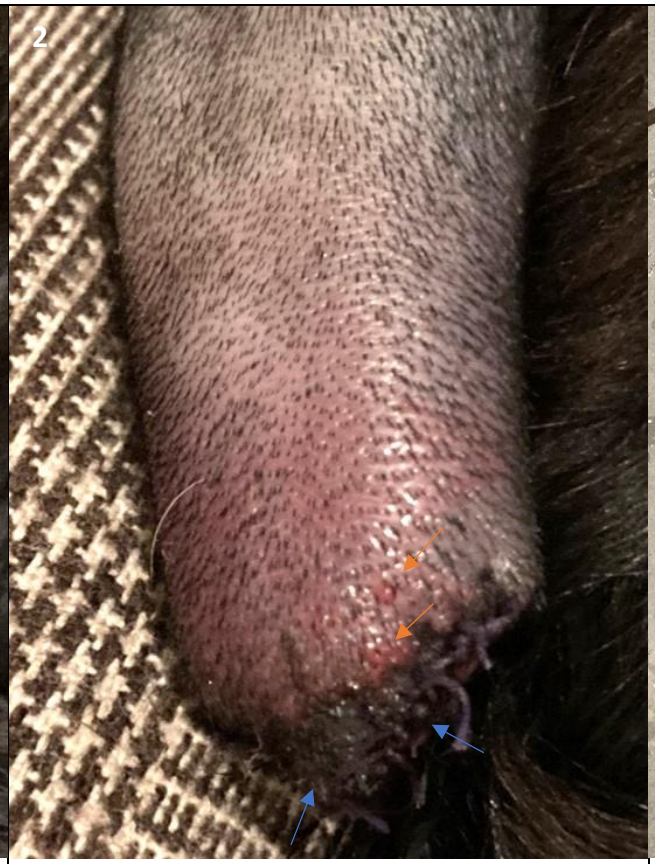
The infection was caused by an infective agent producing large amounts of red-pigmented toxins as part of its assortment of antagonistic tools. To remove the infection, the immune system needed to capture and expel these toxins as well as the debris generated and disseminated in the tissue by the infection in order to prevent them from causing further harm to the dog's tissue. This process was essentially what would determine the speed of healing. Its duration would be relative to the time the infection had had to spread cranially and to settle in.

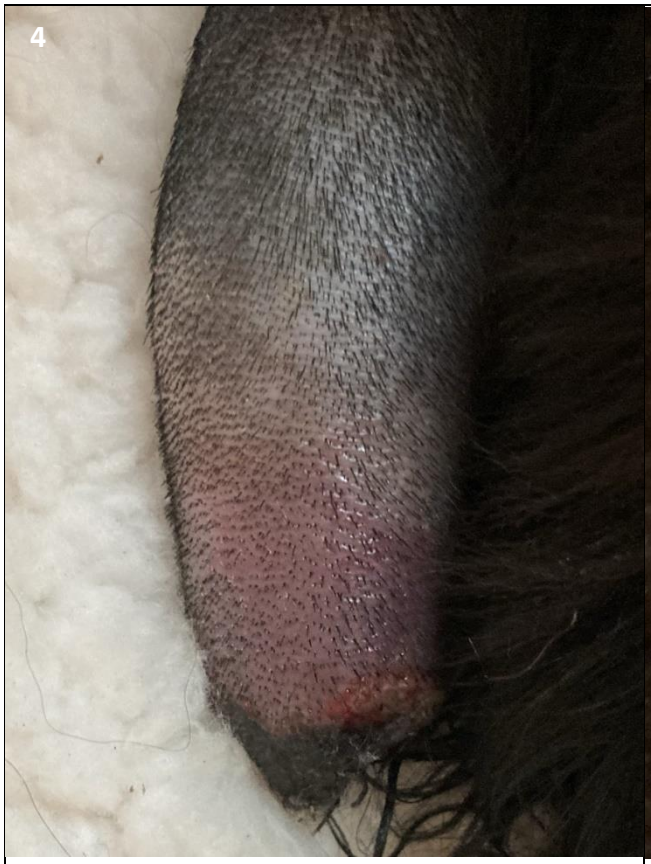
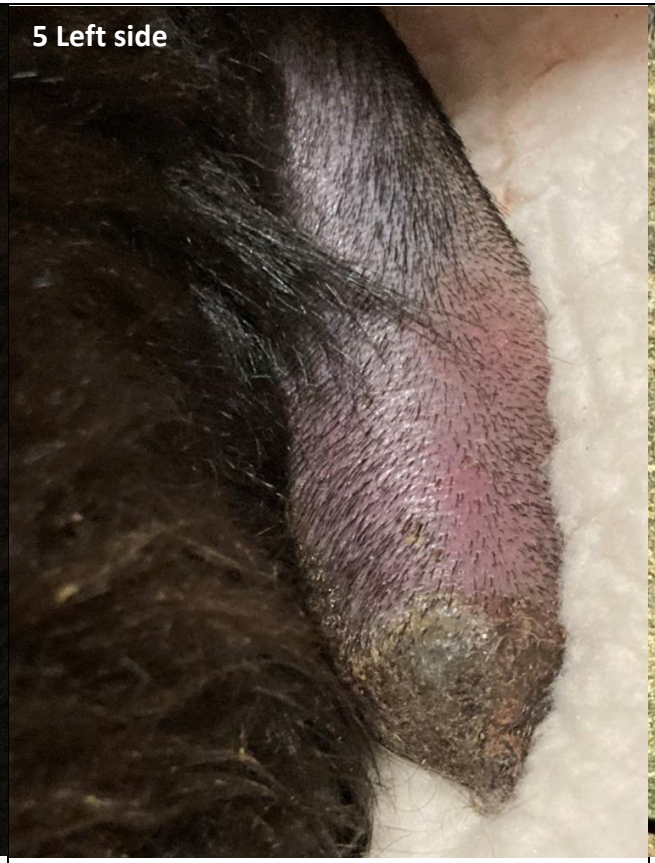
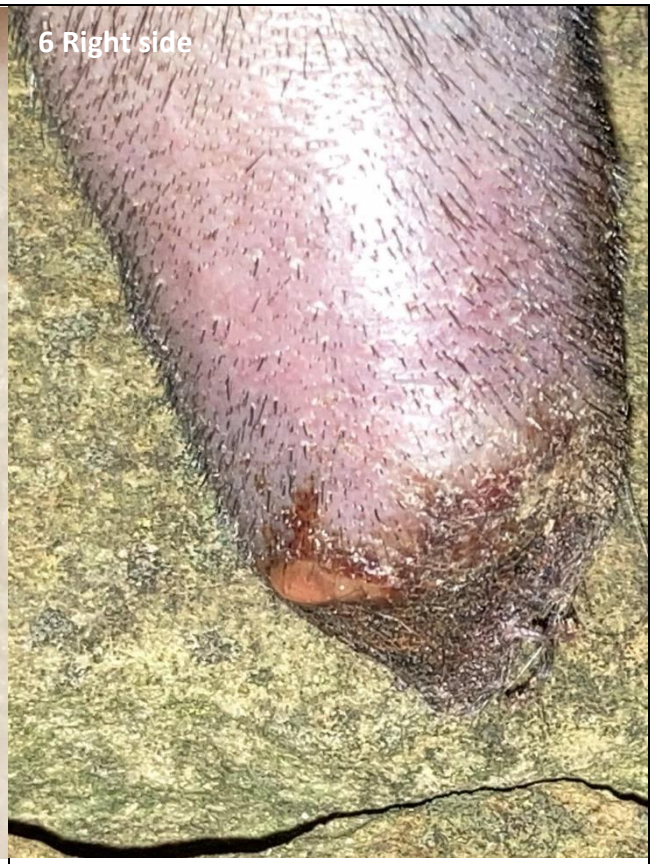
The immune system would lead the captured toxins and other debris to the wound surface where it would leave it to dry and be washed off daily. Sometimes, the toxins are concentrated inside abscesses, and the fact that the immune system has to penetrate each abscess and clean out its contents explains why toxins and other collections of debris, in the later stages of healing after the non-incapsulated toxins have been cleared out, often appear intermittently on the otherwise healing wound surface. The immune system will usually bust one abscess at a time, to always keep control of the situation.

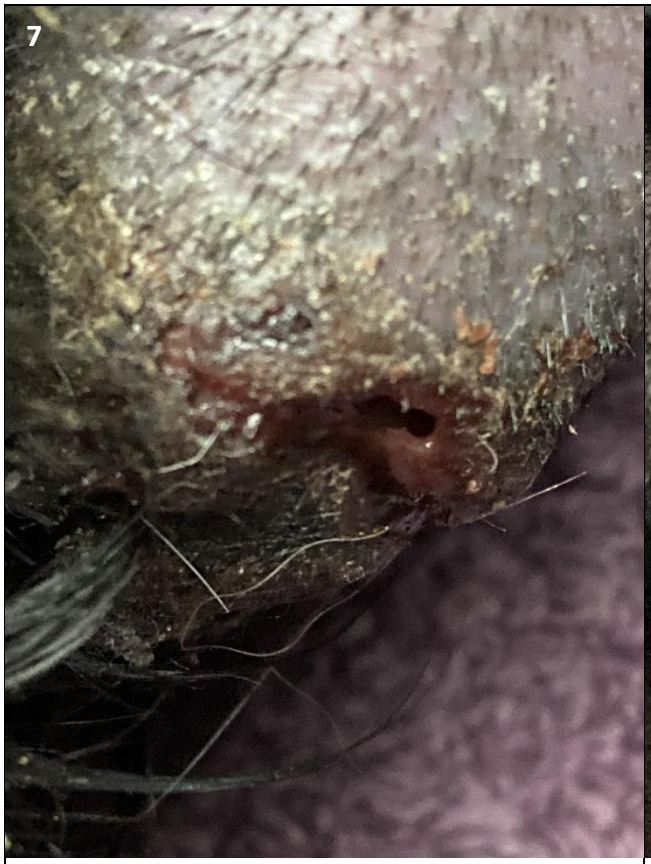
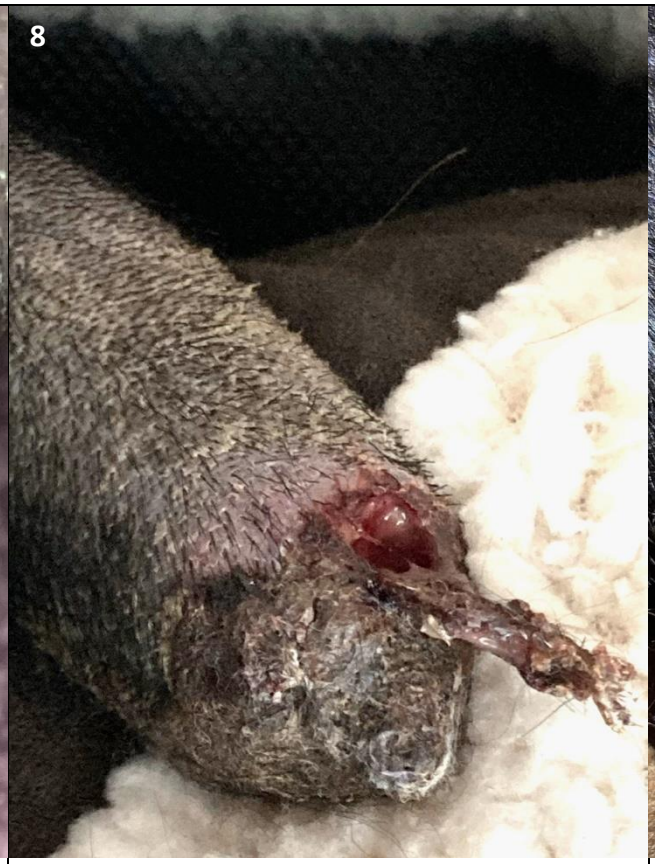
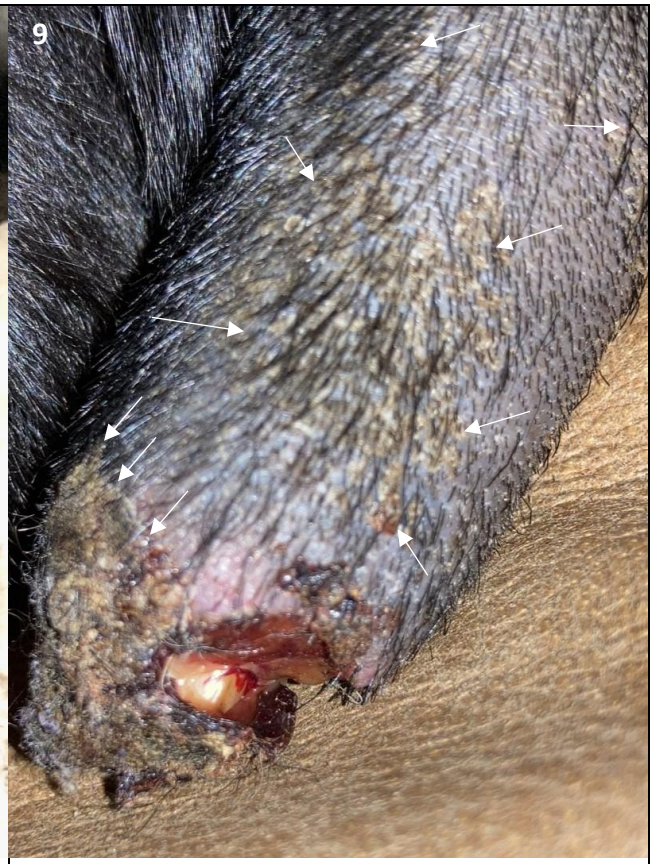
The infection had caused most damage to the ventral side of the tail, which had been covered with the big area of eschar (pic 13 and 14). The eschar gradually softened and fell off revealing intact but still recovering skin underneath. (pic 16, 17, 19, 20, 21 and 24). The damaged skin along the entire stump gradually underwent repair and the border of the infection could be followed as the redness retreated distally (e.g. pic 24, 25 and 26). The skin behind the retreating infection continued to undergo repair and new skin was created. Two weeks after starting MPPT treatment, all soft tissue had been covered with new epithelium (pic 27 and 28). The bone and bone marrow were covered with soft tissue granulation but not yet epithelialized.

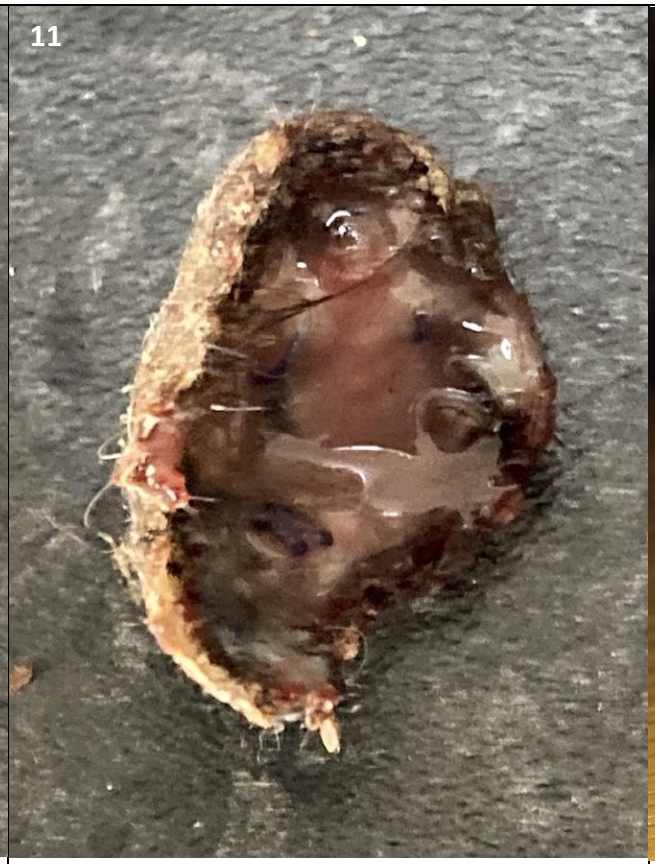
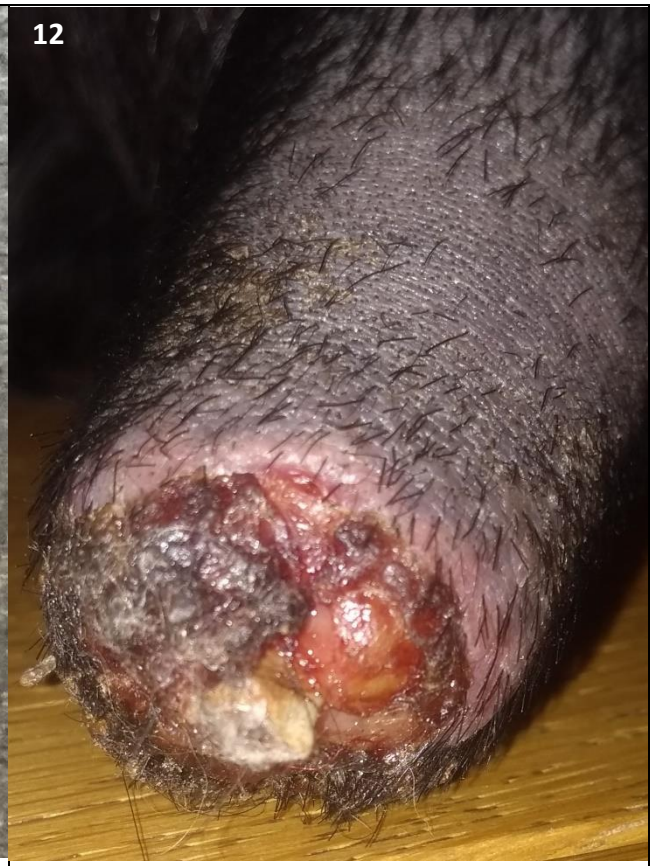
As the amputation had not been performed between vertebrae but by cutting perpendicularly through a vertebra, the cut periosteum, the cut internal bone structure of canaliculi and osteocytes and the cut bone marrow had been trapped directly up against the infected soft tissue that had been sutured over it. This infection had been growing and spreading to these highly sensitive structures under the eschar for 2 weeks before the eschar fell off (pic 12). This is presumably what had affected the bone structure and caused the spread of the infection to the bone marrow, as well. Infection in the bone and bone marrow can cause major complications. This part of the infection was combatted in a similar way to the soft tissue and the toxins were expelled onto the surface of the bone marrow (pic 20, 21 ,25, 30, 32, 33, 34 and 35).

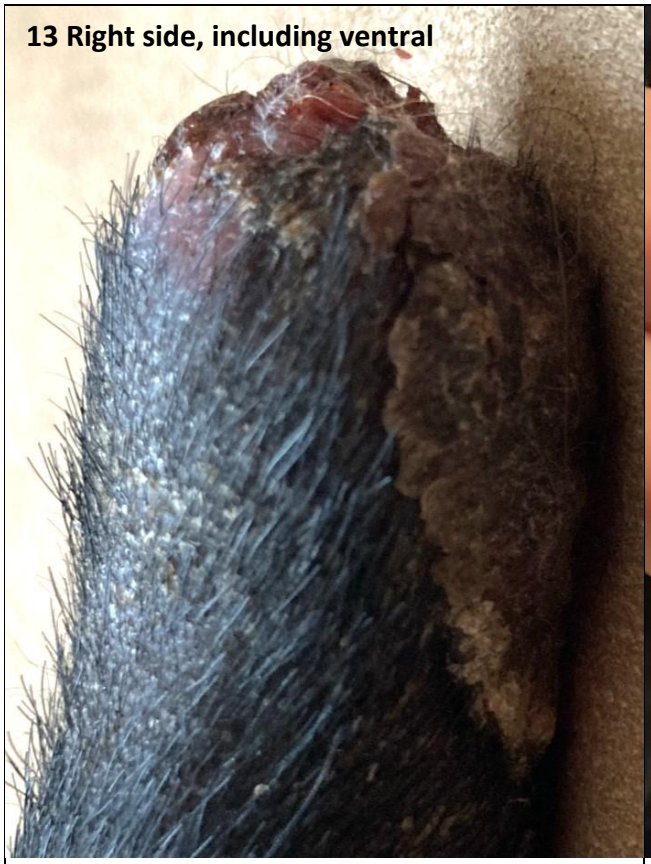
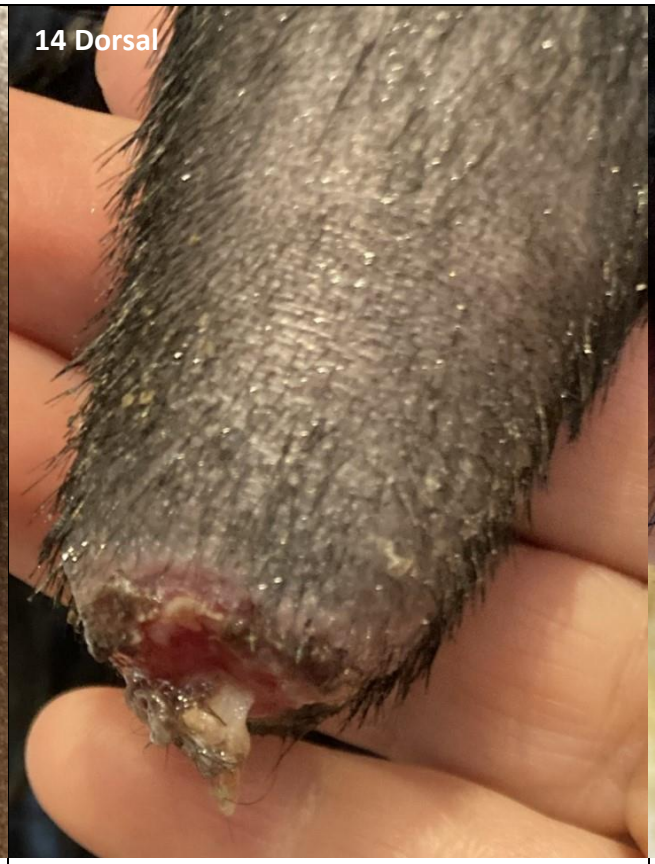
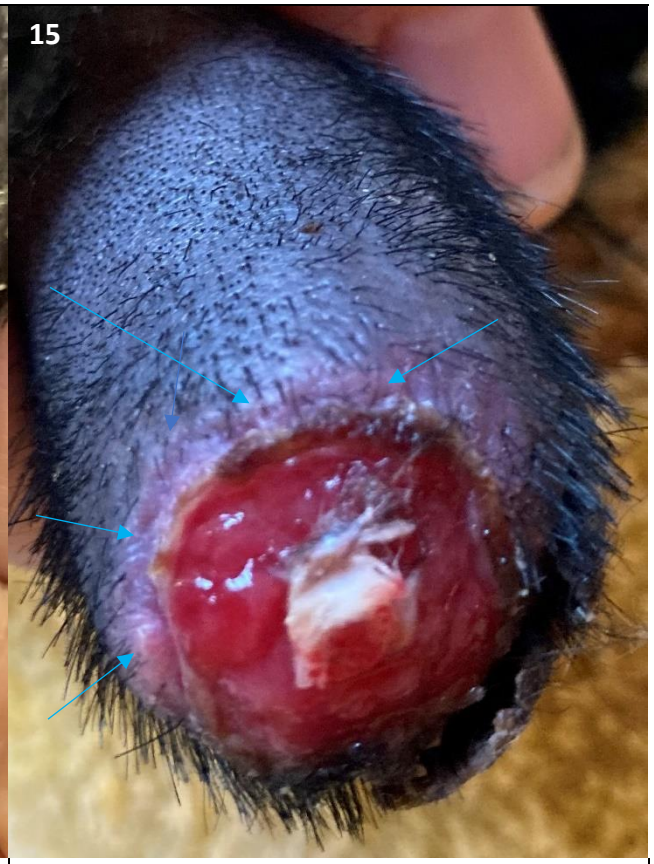
The overall strategy of the immune system seemed to be one of clearing infection, protecting all tissue types, and healing simultaneously. It would clear the bulk of the infection, epithelialize as far as possible whilst still keeping just enough granulating tissue surrounding the bone open to allow for easy expulsion of the remaining bacterial debris (pic 15 to 26). Simultaneously, it was also clearing the bone tissue of infection and, only once this had been achieved, would the granulation tissue expand to cover the severed end of the bone (pic 26, 27 and 28). The next step was to cover the granulation with new epithelium (pic 29), and this progressed at the same speed with which the last toxins were expelled from the bone marrow through the granulation (pic 32 and 33). The tail was fully closed 4 weeks after start (pic 34). The last tiny specs of toxins were still being expelled after full epithelialisation (pic 34 and 35). This soon subsided, indicating that all infection had been combatted, and the epithelium at the distal end matured well (pic 36 and 37). The same was the case for the damaged skin along the tail which regenerated with full pigmentation and restoration of hair follicles (pic 23, 38 and 39). The closure was stable and a month later the tail was fully covered and protected by healthy, shiny regrown hair (pic 39 and 40). A follow-up two years later showed a fully recovered tail which had had no issues at all after closure (pic 41 and 42).

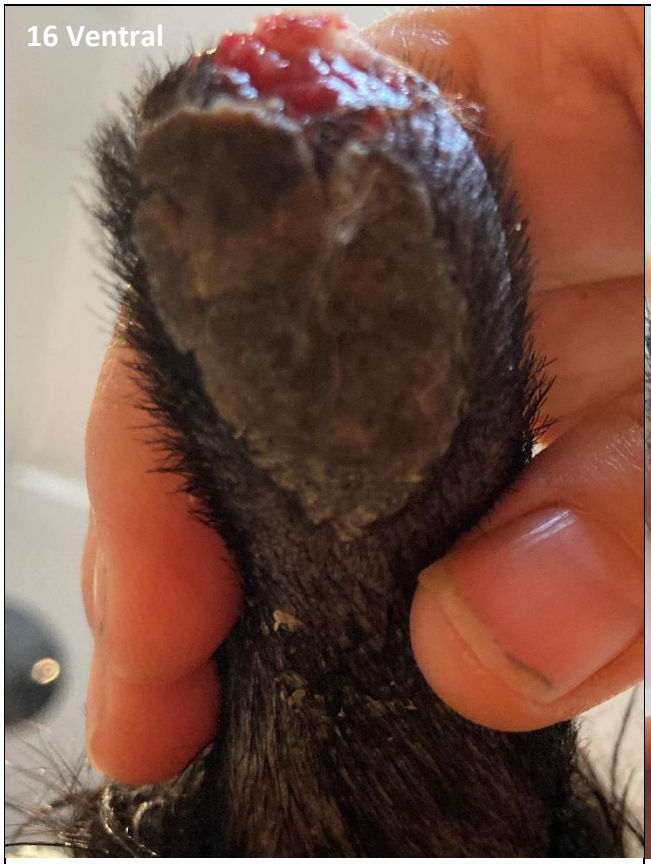
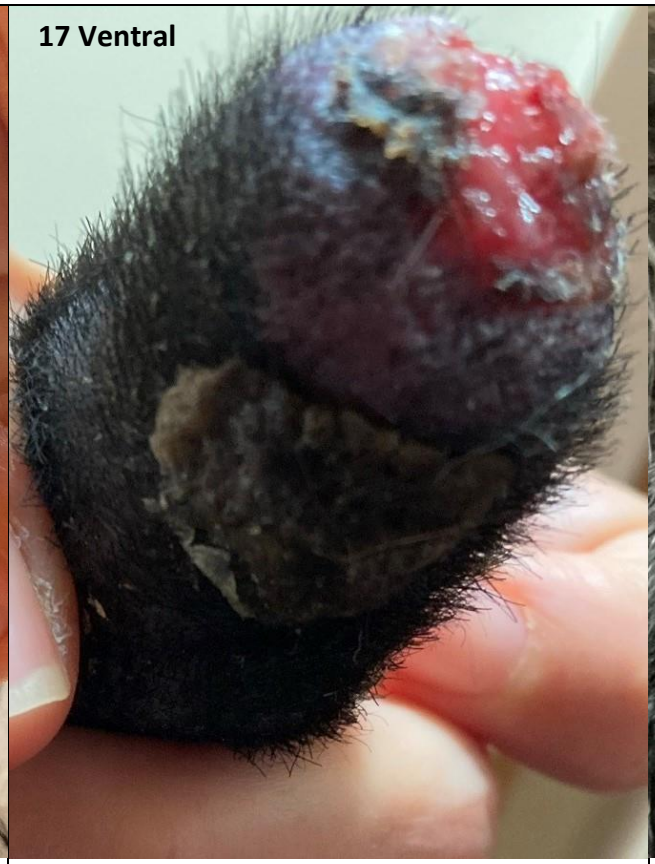
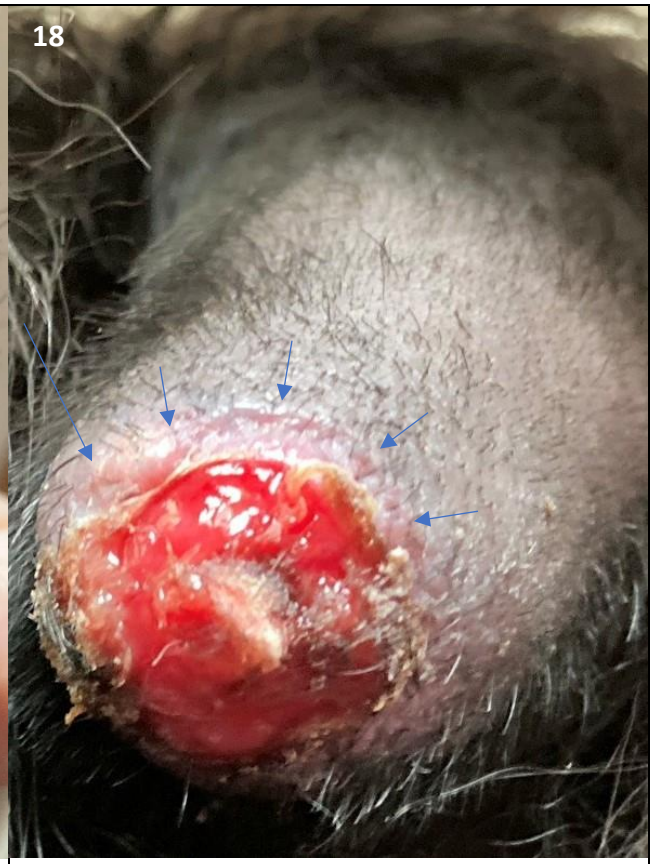
		
Day minus-14 **Day of amputation** **2 weeks before first MPPT** Amputation	**Day minus-13** **1 day after amputation** 24 hours after the amputation. The distal 20mm of the tail are inflamed with drops of liquid oozing through the intact skin. The black matter building over the sutured site is necrosis, not scab. *Orange arrows*: Droplets of oozing liquid *Blue arrows*: Dry necrosis building (eschar)	**Day minus-11** **3 days after amputation** Necrotic discharge exiting between the stitches of the amputation and falling on the floor.

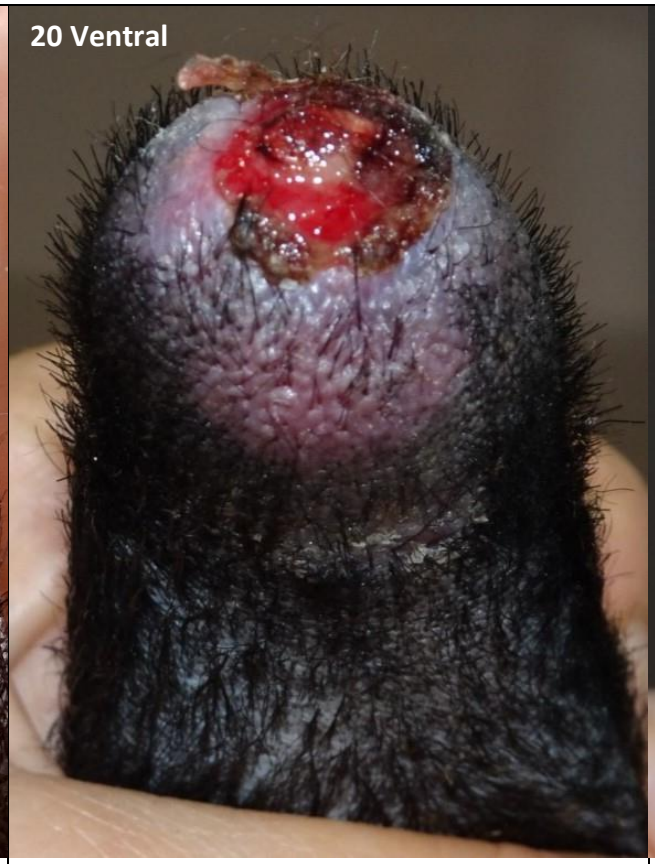
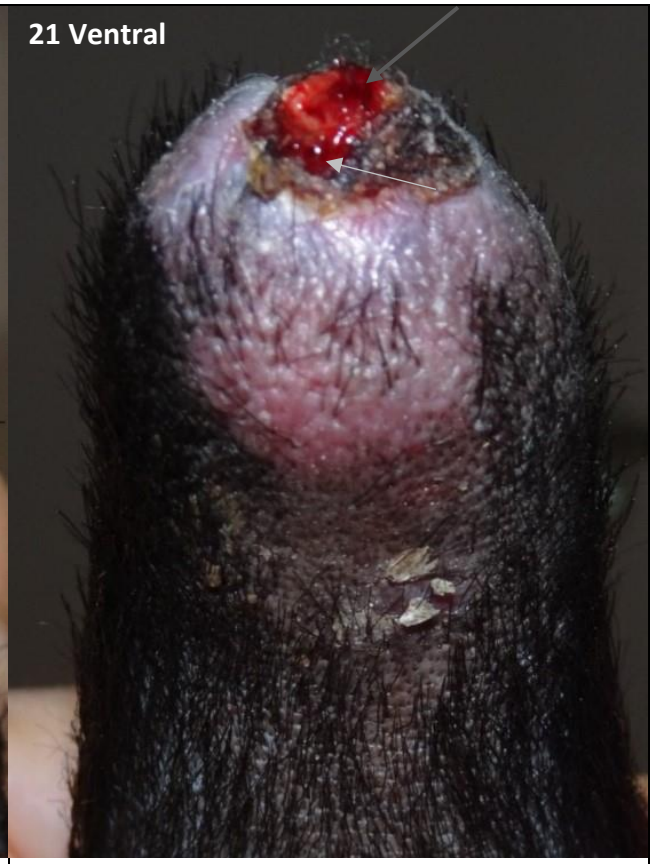
 4	 5 Left side	 6 Right side
Day minus-11 **3 days after amputation** It is not clear from the outside that the infection has spread much further interiorly than what was evident from the outside 48 hours prior. This is becoming clear from the portions of necrotic debris discharging from the amputation site. (See pic 3).	**Day minus-9** **5 days after amputation** The inflammation is spreading cranially at pace. The sutures are covered with a thick eschar. The eschar is being mistaken for a scab.	**Day minus-9** **5 days after amputation** Infectious debris is seeping/exuding from under the eschar.

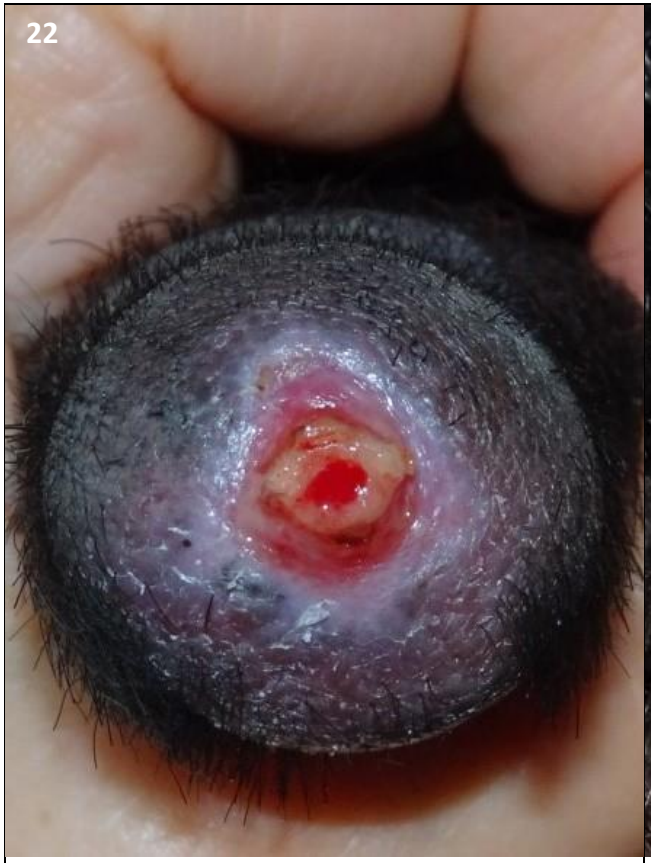
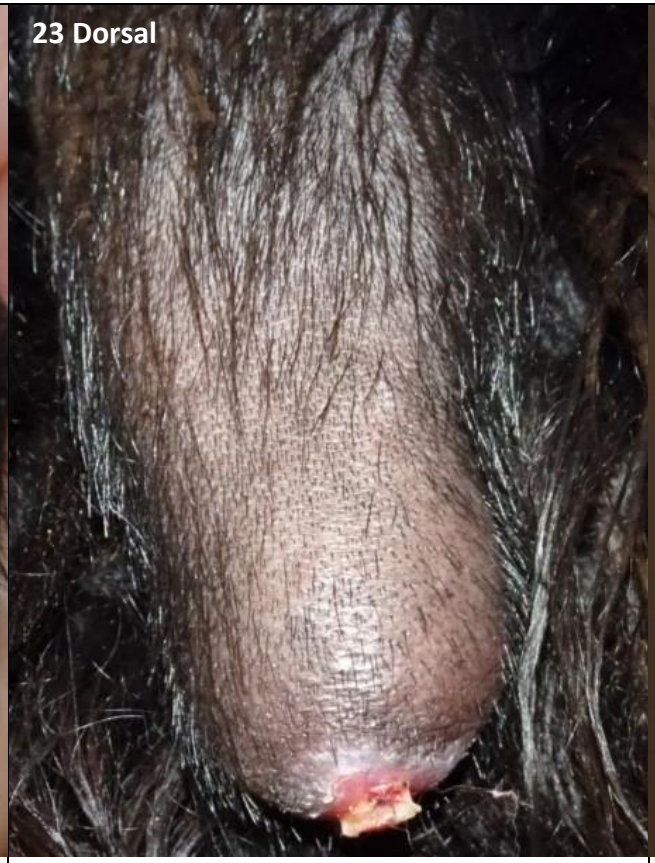
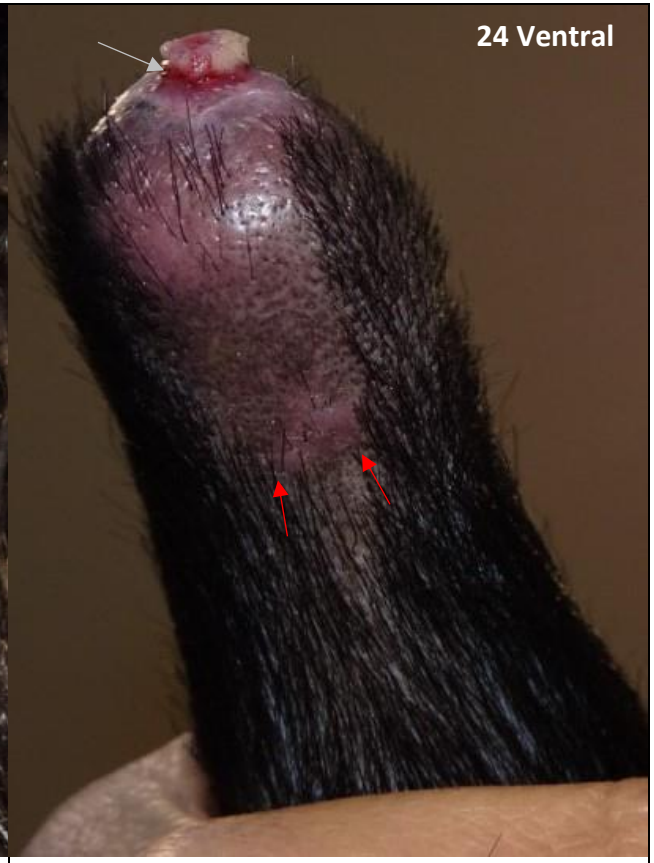
		
Day minus-8 **6 days after amputation** 18 hours with systemic antibiotics. The seeping infectious debris is changing to a higher viscosity which retains a higher proportion of it in the soft tissue, where it can cause further harm.	**Day minus-6** **8 days after amputation** Two days with systemic antibiotics. The infectious debris is drier and more viscous. The place of the former seepage between the tissue and the eschar is dry and hollow. The necrotizing processes continue cranially inside the soft tissue, but the debris is not readily released. It is released under the eschar causing this to grow, and through the intact skin along the length of the tail as a dry, grey-ish, shedding, outer layer. Reduced redness and swelling.	**Day minus-3** **11 days after amputation** The soft tissue is being consumed under the eschar. The site is dry and the debris is retained in the soft tissue. Dry necrosis is moving up along the tail. *White arrows:* big necrotic patch of eschar growing at the end of the tail and along the ventral side, as well as smaller ones appearing as scales in the skin along the length of the tail.

10 	11 	12 
Day minus-2 **12 days after amputation** Outside view of the 15-20 mm thick, intact, strong cap of eschar which has fallen off the site of surgery.	**Day minus-2** **12 days after amputation** Viscous infectious debris on the inside of the cap of eschar. As it dries out, it adds to the thickness of the cap.	**Day minus-2** **12 days after amputation** The appearance of the amputation site under the eschar. No sign of the stitches. The soft tissue is infected and suffering dry necrosis with no signs of healthy tissue. This has left the bone sticking out approximately 5mm. The bone tissue and the bone marrow are different tones of grey, indicating necrosis. The skin is retreating, and the hair follicles are disappearing along the tail, indicating deeper damage to the skin.

13 Right side, including ventral 	14 Dorsal 	15 
Day 0 **14 days after amputation** A big patch of dry eschar is covering the infection 4-5 cms up the tail ventrally. The dorsal side is covered with small scales of dried out necrosis. The hair follicles are disappearing.	**Day 0** **14 days after amputation** **Just before first MPPT treatment** 5 mm of grey, dull infection-affected bone with necrotic tissue attached despite thorough cleaning with water and removal of loose tissue in preparation for MPPT application. The wound edges are dull and lifeless without signs of epithelialisation. Soft tissue is visible but dull and without granulation. The hair follicles of the intact skin along the tail are disappearing.	**Day 1** Wound bed is clean and fully granulating. Wound edges are epithelializing. The external surface of the bone is undergoing cleaning/autolytic debridement. A necrotic outer layer is being peeled off. This is required to ready it for soft tissue coverage. *Blue arrows*: New epithelium.

		
Day 1 Eschar is loosening along its borders. Strong soft tissue granulation.	**Day 2** A piece of eschar has disappeared.	**Day 2** The colour of the bone tissue is changing into ivory. The bone marrow is changing into red. Healthy granulation. Wound edges are epithelializing. Wound area is reducing. *Blue arrows*: The border in front of which the epithelium is new, i.e. generated within the past 48 hours.

		
Day 3 Granulation is covering more of the bone with only 2 mm sticking out. The bone colour is ivory with a more viable appearance. The bone marrow is pink/red. More eschar has disappeared and the thinner scattered patches of dried-out eschar along the tail are being disposed of. The skin appearing underneath is red and under repair.	**Day 5** All dry necrotic eschar on the skin is disappearing. Underneath is intact skin – but not fully healthy yet. Infectious debris continues to be disposed of through the wound opening. Despite this, light grey new epithelialisation continues to reduce the size of the opening. The bone marrow has a dark red convex coverage of expelled toxins drying across the surface.	**Day 8** Light grey band of epithelialisation is reducing the size of the wound opening. Red toxins mixed with other debris are carefully being deposited in the opening remaining around the bone. Similarly, toxins are being expelled from the bone marrow. *Dark grey arrow*: Drying droplet of red bacterial toxins expelled from the bone marrow. *Light grey arrow*: Droplet of red bacterial toxins expelled from the soft tissue.

 22	 23 Dorsal	 24 Ventral
Day 11 morning Grey tissue: maturing epithelium Pink tissue: granulation Ivory: Bone tissue with red bone marrow in the centre.	**Day 11 evening** The inflammation is gone from this dorsal view and from the right side of the tail. The swelling is still present in the left side. Hair follicles are missing n some areas. The opening has practically epithelialized, thereby protecting the soft tissue as far as possible whilst still allowing the escape along the bone of toxins from the soft tissue. The bone is undergoing cleaning and repair of the periosteum as required for its coverage with granulation tissue. The band of whitish grey border is new, maturing epithelium.	**Day 11 evening** The big sheet of eschar is gone. The red border shows the progress of the ongoing distal retreat of the infection. The grey non-swollen skin cranially to this border has been cleared and the red, stretched skin distally covers the area that is still under pressure from the soft tissue infection underneath. The bone is whitish/ivory and recovering and the bone marrow is red and recovering. Only a narrow band of soft tissue wound is still open and is serving as site for the expelled red toxins. *Red arrows*: Clearly demarcated border between infected and non-infected part of the tail. *Grey arrow*: Band of expelled red bacterial toxins.

Day 12

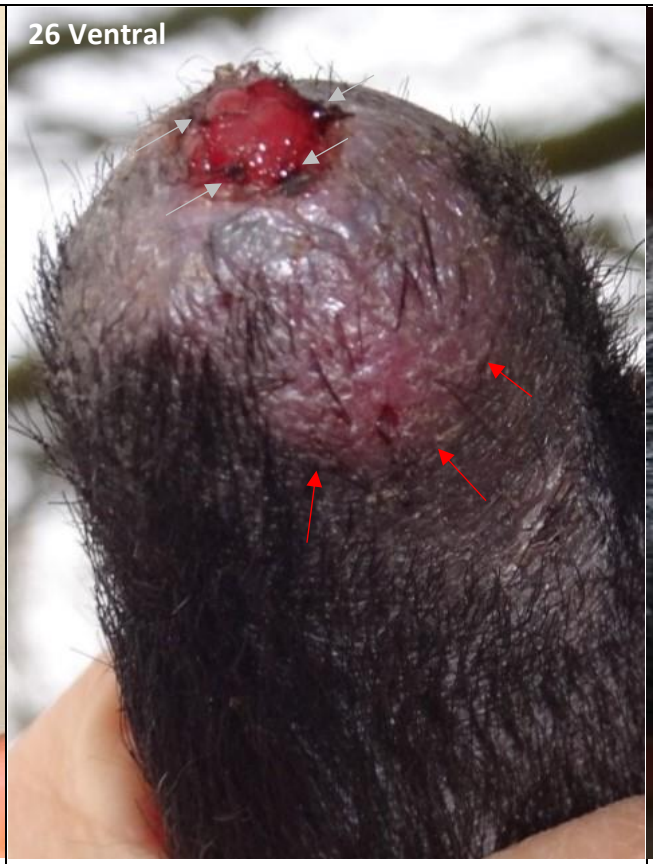
The pressure under the skin has decreased. The skin even looks “deflated”. Toxins and debris are being expelled. The bone is still sticking out. Red toxins are also being expelled from the bone marrow.

Red arrows: Demarcated border between infected and non-infected part of the tail.

Light grey arrow: Droplet of red bacterial toxins expelled from the soft tissue.

Black arrow: Red bacterial toxins expelled from the bone marrow.

Orange arrow: Droplet of expelled yellow bacterial toxins.



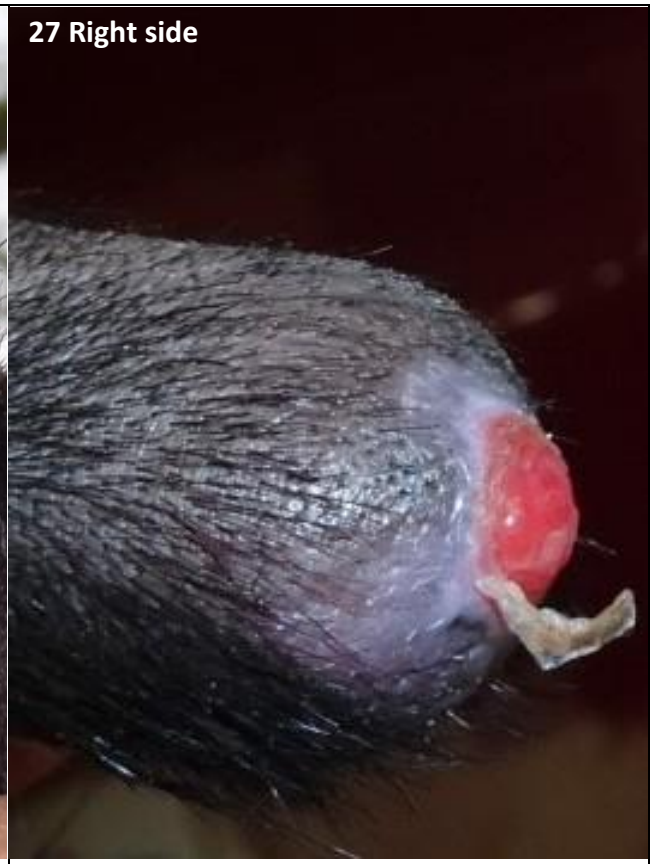
Day 13

The bone has been covered with granulation tissue and the swelling and redness have further reduced.

A few spots of toxins are visible on top of the soft tissue, at the edge of the epithelium, next to the bone.

Red arrows: Border between infected and non-infected part of the tail.

Light grey arrows: Drying droplets of red bacterial toxins expelled from the soft tissue.



Day 14

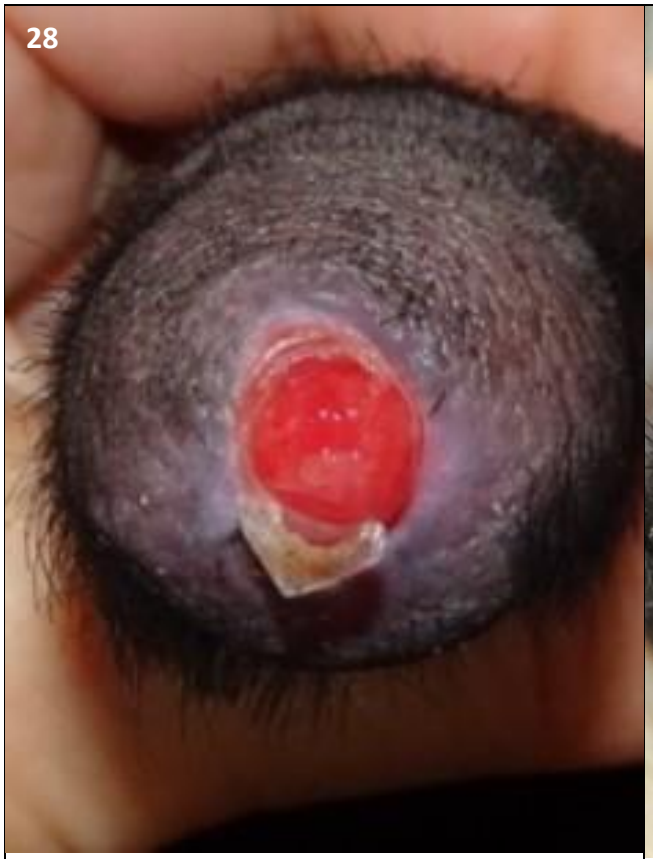
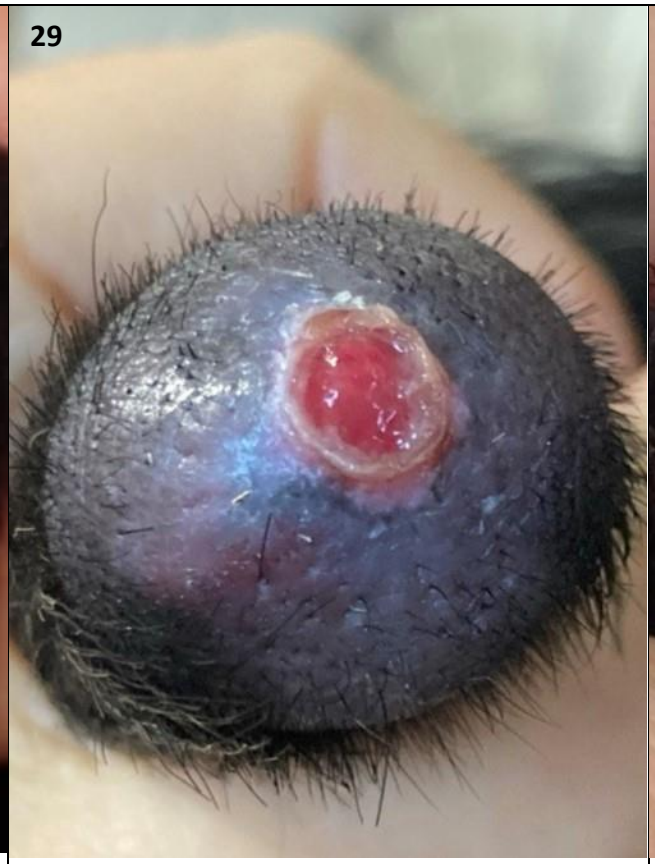
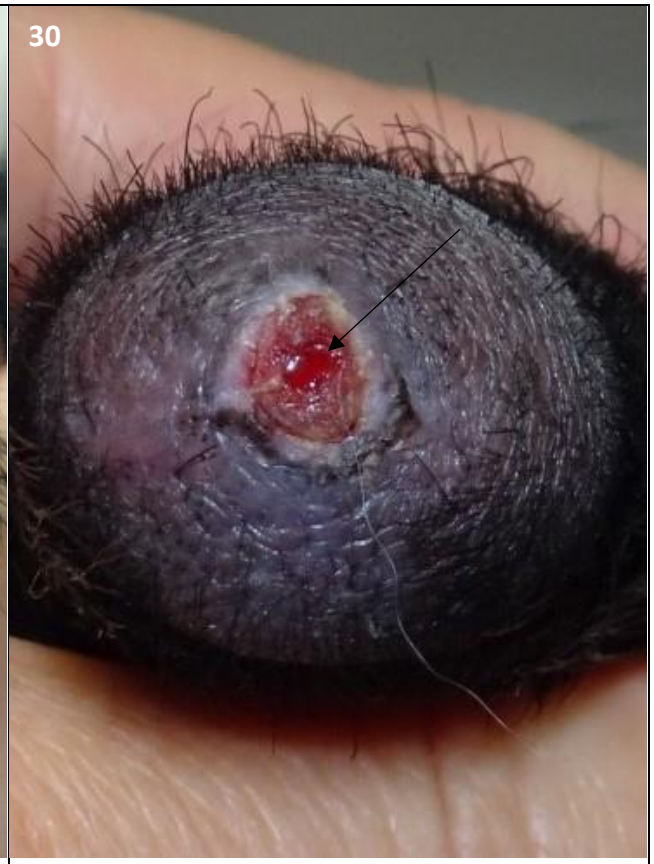
No signs of redness or swelling.

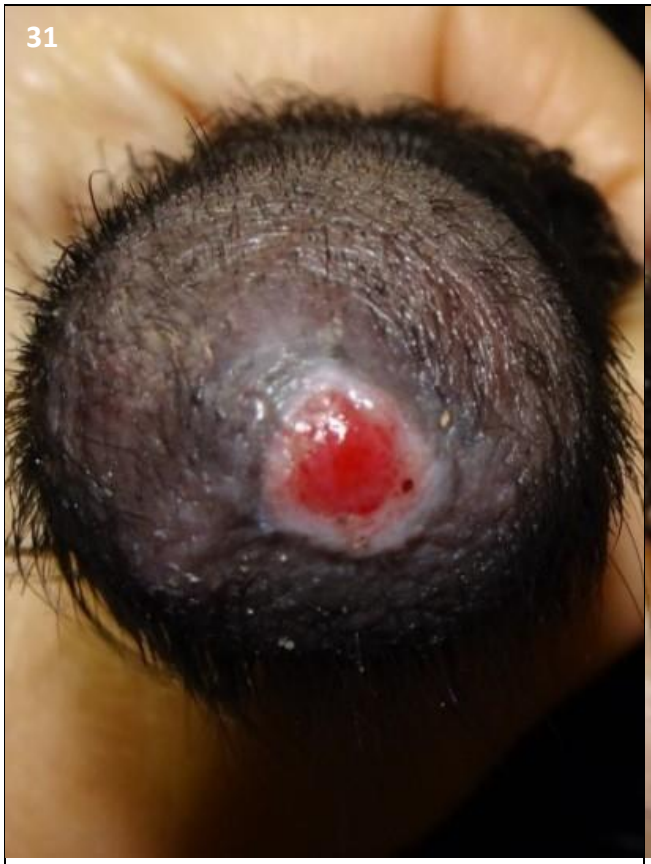
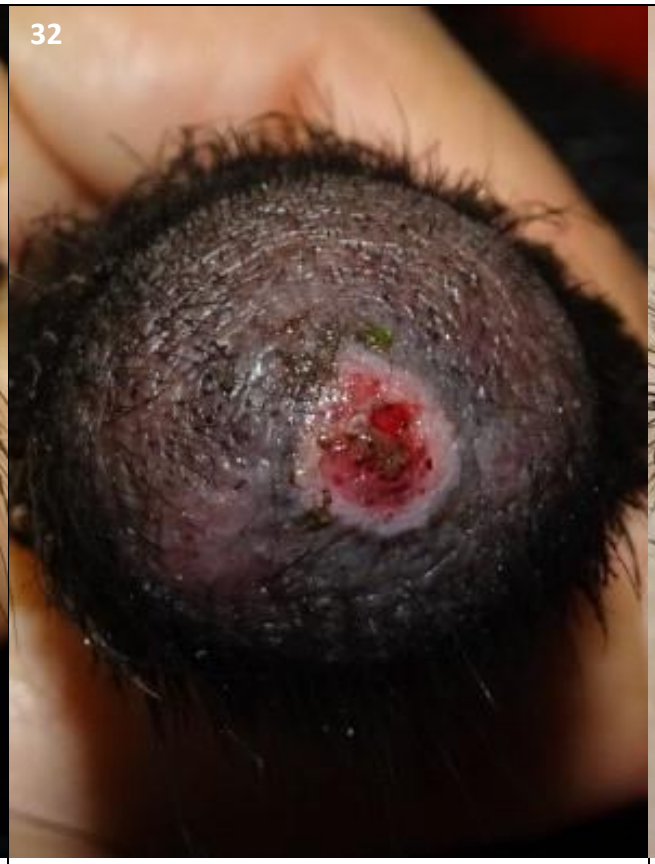
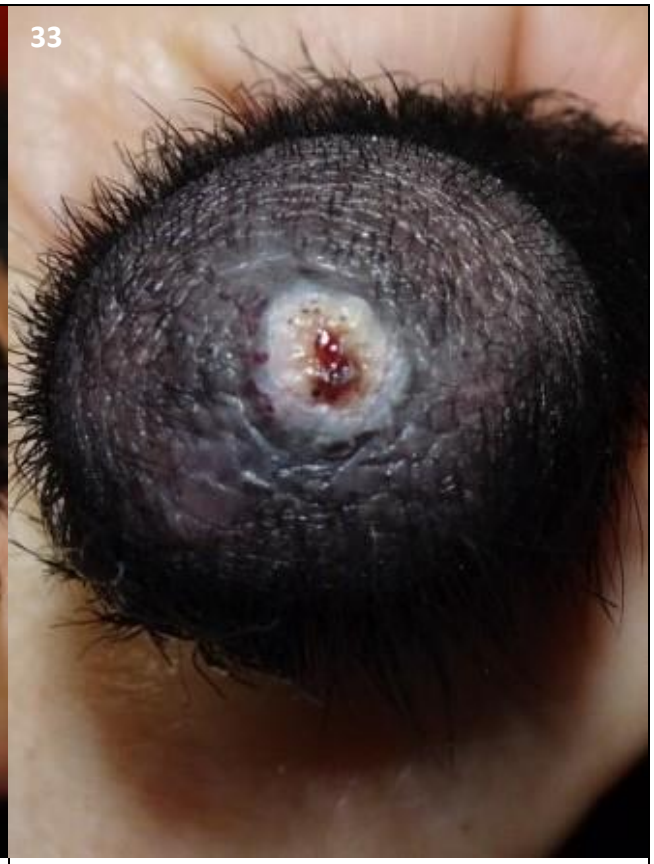
New epithelium is covering all soft tissue. Healthy pink granulation tissue covering, cushioning and protecting the bone underneath.

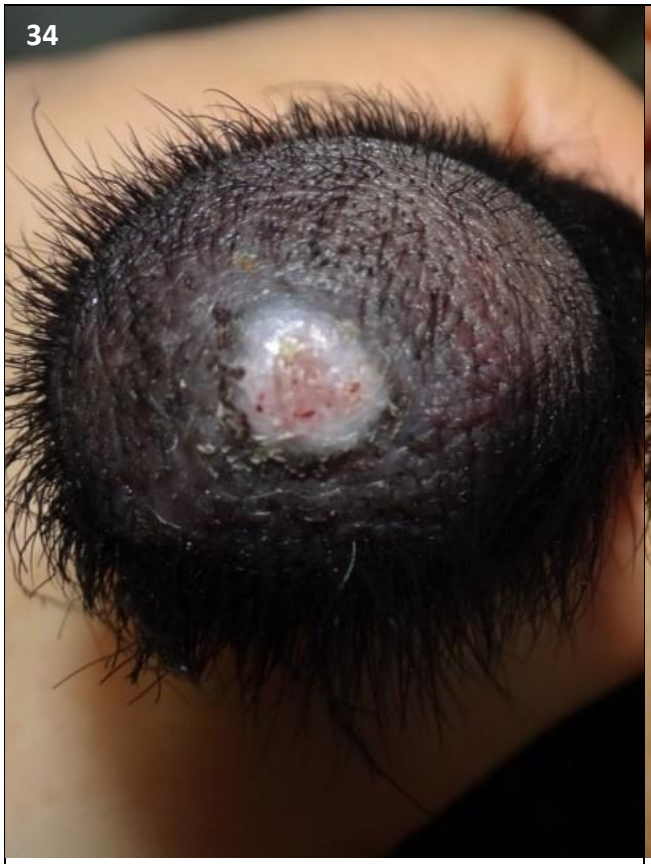
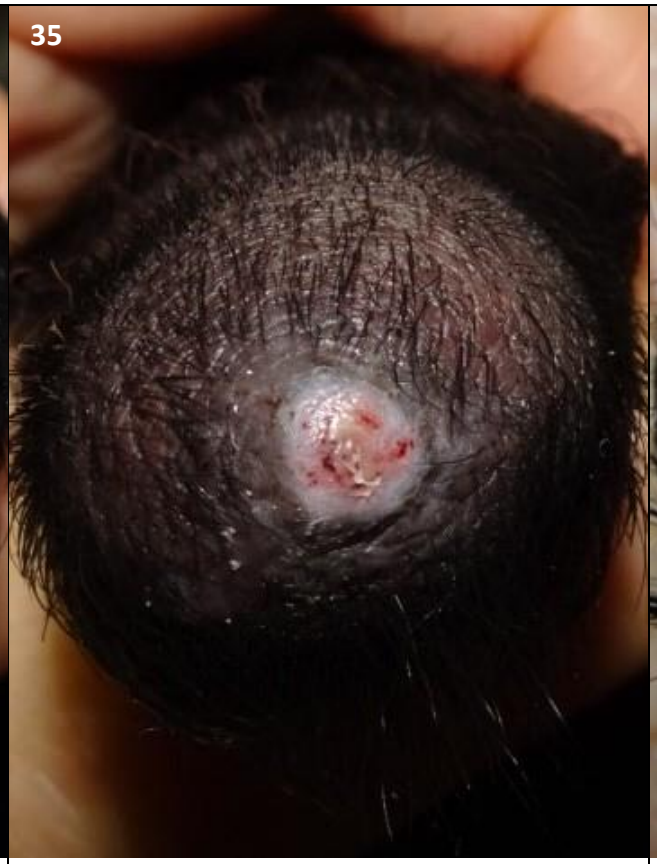
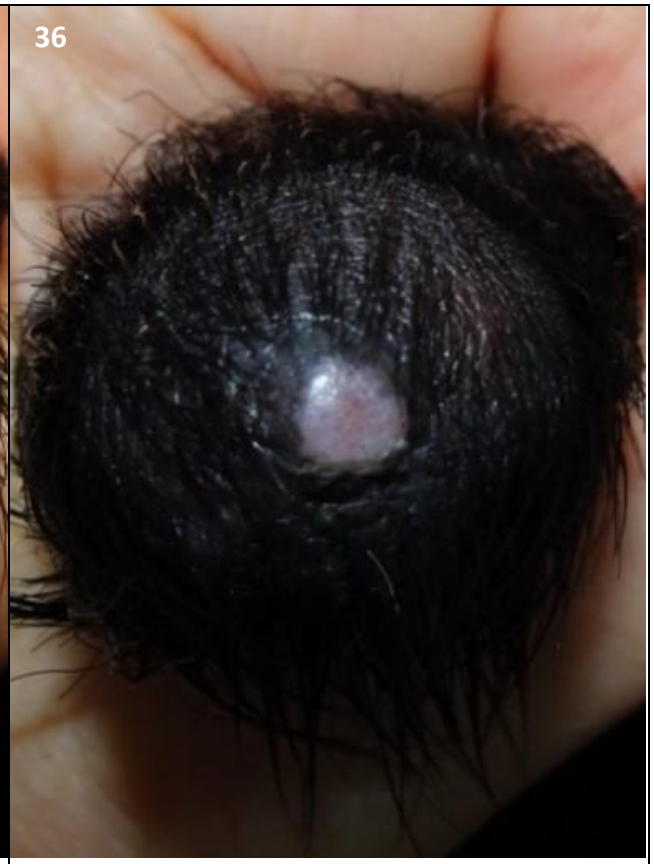
The light grey shade is new healthy skin cells still maturing.

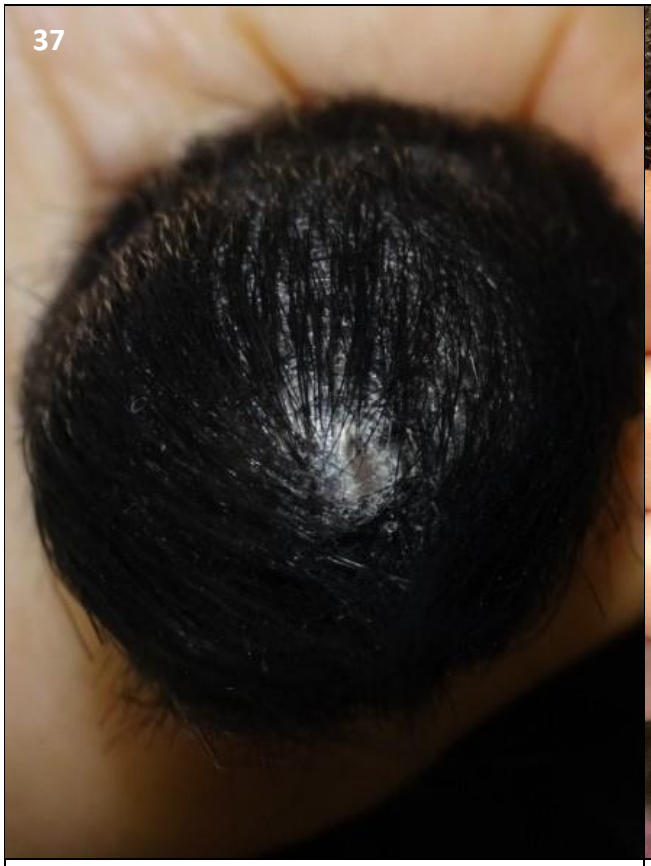
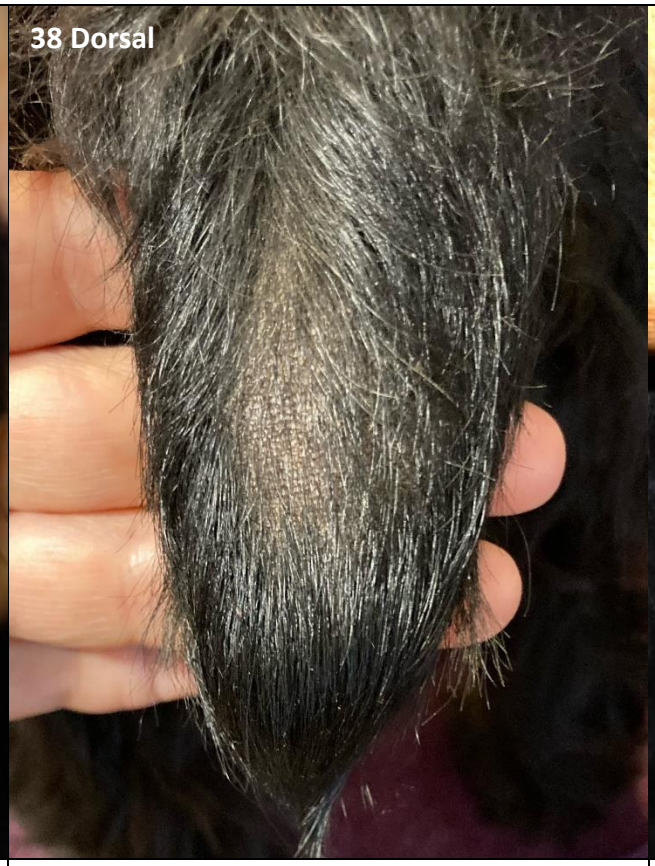
The light brown band is the protection underneath which epithelial cells are created and protected until they are ready for maturation in the presence of air and the band is dismissed.

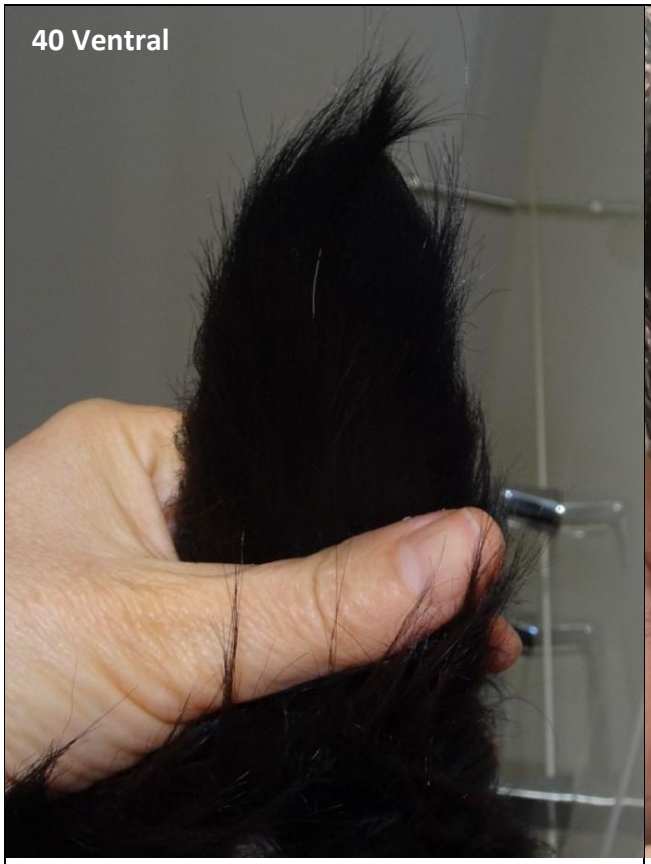
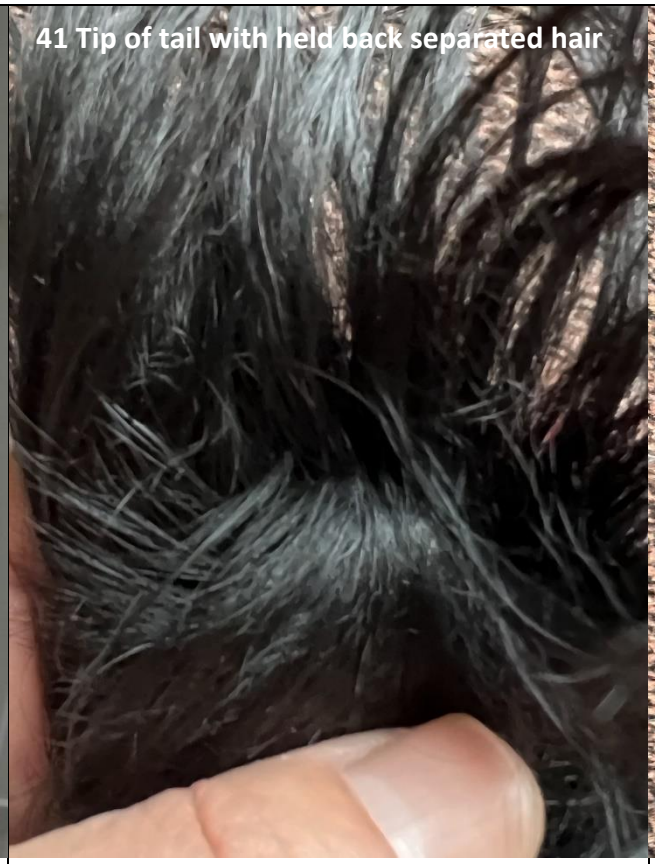
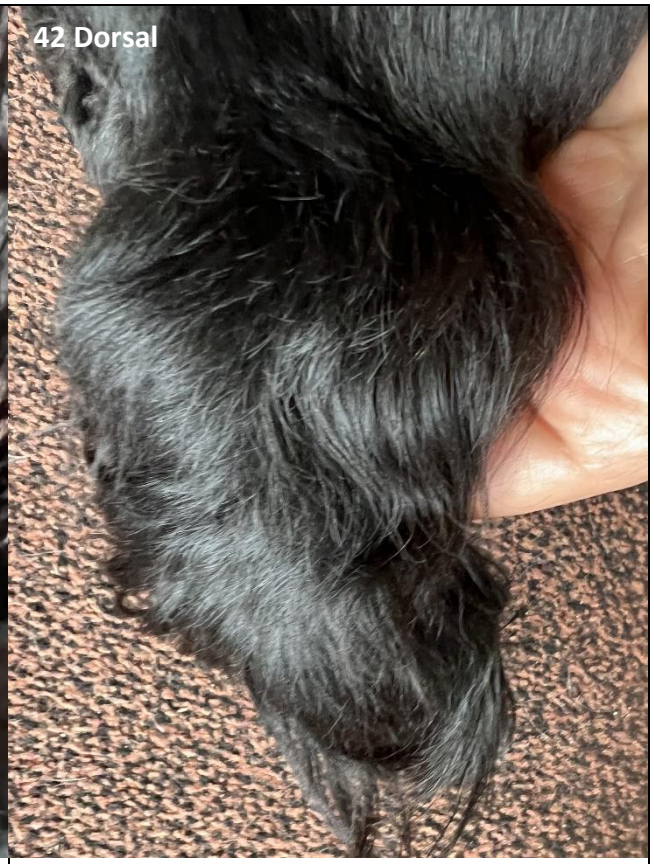
Hair follicles are reappearing in the skin along the tail, as a sign of full skin recovery.

28 	29 	30 
Day 14 No signs of redness or swelling. All soft tissue has fully epithelialized and is maturing. The bone and bone marrow are fully covered with pink healthy granulation, serving as protective cushioning. The light grey shade is new, healthy, still maturing skin cells. The light brown band is lifting as a sign that the epithelium is sufficiently mature to let go of this protection and ready to mature in the presence of air. Hair follicles are reappearing in the skin along the tail, as a sign of full skin recovery.	**Day 18** Epithelialisation of the granulation covering the bone. The soft, very delicate brown border is the advancing protective film underneath which the epithelialisation takes place and covers the new epithelial cells until they are ready to mature without this protection.	**Day 20** The granulation covering the bone and the advancing epithelializing protective film covering the synthetisation of the epithelium has disappeared. Judging from the skin edges, it occurred accidentally by the outside world. Epithelium is surrounding the bone on all sides perfectly outlining the end of the severed bone. Toxins continue to be expelled from the bone marrow. *Black arrow*: Droplet of red bacterial toxins.

31 	32 	33 
Day 21 Granulation is again covering the bone and bone marrow. The light grey circle is maturing epithelium. The fragile brown film protecting the synthetisation of the epithelium (see pic 29) has not been restored.	**Day 22** Toxins continue to escape through the newly formed granulation tissue covering the bone and bone marrow. All soft tissue of the tail has epithelial coverage.	**Day 27** Epithelium is covering the bone and the remaining toxins are escaping from the bone marrow only. All bone tissue has epithelial coverage.

 34	 35	 36
Day 28 **Closed** Epithelialisation has been completed. Bone marrow has epithelial coverage.	**Day 30** Small specs of toxins are still being expelled through the epithelial layer.	**Day 35** The epithelium is thickening and maturing well. No signs of any more toxins.

		
Day 41 The scar is ½ x 2 mm. The hair follicles and pigmentation of the new skin and the repaired skin have been fully restored.	**Day 51** Hair follicles are being regenerated. Hair from these follicles is still very short and the fur therefor still appears thin.	**Day 73** **Follow-up 6 weeks after full closure** All hair has been fully restored and is shiny and healthy. The stump is well protected.

 40 Ventral	 41 Tip of tail with held back separated hair	 42 Dorsal
Day 73 **Follow-up 6 weeks after full closure** The amputation site is impossible to see. The stump is fully protected by hair.	**Day 728** **Follow-up after 2 years** It proved impossible to identify any scar at the tip of the tail which was entirely and abundantly covered with hair.	**Day 728** **Follow-up after 2 years** The dog “made a complete recovery and has had no issues at all since with the tail.”