Twice amputated tail with AMR infection, including in the bone (osteomyelitis).

Springer Spaniel Female 3 years 4 months old

"Day 0" equals the first day of MPPT treatment

This 3-year-old female Springer Spaniel (undocked) had damaged her tail from happy wagging and associated banging. This was a regular occurrence (more or less weekly) and the tail had become infected and very sore (pic 1). Two days later (pic 2, 3), the vet scheduled her for a partial tail amputation a whole week later and in the meantime prescribed oral pain medication, Meloxicam (NSAID) 16.5 units oral once daily (Metacam).

6 days later the dog underwent the tail amputation, as scheduled, and was given 7 days antibiotics 400mg Amoxicillin+100mg Clavulanic acid (Clavudale) twice daily and to continue on Meloxicam. She also received several sessions of laser therapy following surgery.

The day following surgery, the tail was bleeding and one stitch had opened. The tail was washed and rebandaged by the vet.

Two days after that, the tail was bleeding through the bandage. The loose stitch was removed. The tip of the tail was inflamed with necrotic patches and felt icy cold. These are signs of infection and deterioration. It could therefore be concluded that the infective agent was resistant to the antibiotic provided. However, the antibiotic was not changed but the course (Amoxicillin & Clavulanic acid) prolonged for another 7 days. Instead of a bandage, the tail was covered with a plastic mesh material ("Dog End").

Three days later, and again three days later, the wound was re-meshed by the vet. The amputation site was necrotic with no signs of granulation or epithelialisation and the skin displayed clear sings of the necrosis spreading further cranially. There was no swelling. but the necrosis had already eaten away the tissue in which the stitches were attached leaving all the stitches loose with some even dangling (pic 4).

At reinspection the following day, the wound was exposing the amputated bone and a further amputation was carried out on the same day. The technique performed used bone cutter through the body of a vertebra followed by suturing of the soft tissue and skin to cover the bone. The same antibiotic (Amoxicillin & Clavulanic acid) was ordinated for further 4 days. Likewise, Meloxicam was continued, and Gabapentin 100mg 2xdaily was prescribed for 10 days for its analgesic properties.

4 days after the second amputation (pic 5 minus 1 day), the vet found the end of the tail swollen and painful. The soft tissue underneath the stiches was necrotic (black). The tail was dressed with Manuka honey, occlusive dressing and plastic mesh on top. The deterioration continued and 7 days after the amputation, (pic 6 minus 1 day), a 3rd amputation was proposed to try to stop the rapidly spreading, antibiotic resistant infection and prevent it from reaching the dog's trunk. The dog had already undergone 2 unsuccessful amputations and, whether a 3rd amputation could stop the spread, was uncertain. This would most likely be the last possible attempt, as the dog was running out of tail.

The next day (pic 6), the wound was again checked and prescribed another 4 days of the same antibiotic (Amoxicillin & Clavulanic acid - to which the infection was resistant). As the dog was showing signs of excruciating pain, the Metacam was changed to 200mg paracetamol + 4.5 mg codeine (Pardale) 2xdaily in addition to the gabapentin it was already taking. The vet also instructed the owner to apply Manuka honey gel (Kruuse) and hydrogel Intrasite gel) at home.

Over the following two days the tip of the amputated tail continued necrotising and completely detached from the rest of the tail (pic 7, 8). In the centre was a deep, distinct, round tunnel of 2 - 3mm diameter leading to the tail bone (pic 8, 9), suggesting that the infection had spread to the bone and caused osteomyelitis. At no point since the first amputation had the dog lifted her tail voluntarily as it had either been between her legs or hanging down slightly to the side, at an odd angle. At this point in time, however, the dog had become lethargic, presumably due to bacterial toxaemia, and non-engaging due to the associated pain and the toxaemia.

All medication the dog was receiving (1 type of systemic antibiotic and 2 types of analgesics) was now stopped, the necrotic lump and all easily accessible stitches were removed (pic 9) and SertaSil applied to the entire wound bed, inflamed skin and 5-10 mm onto non-inflamed seemingly healthy skin , as if to place a barrier in front of the cranially spreading infection (pic 10).

Within 12 hours, the wound bed showed signs of granulation, angiogenesis, and epithelialisation (pic 11). Within 36 hours the cranial progress of spread was reversed (pic 12) , and over the course of the first week the inflammation along the tail would gradually retreat in distal direction leaving a normal-temperature, non-discoloured, non-swollen tail behind with a granulating wound bed and epithelializing wound edges (pic 12, 13, 14, 15)

The thinning of the skin was reversed and, on the edges of the weak skin, a healthy crust would form (pic 14, 15,). This would protect the ongoing skin regeneration and fall off when the epithelium underneath it was sufficiently mature and ready to let go of its protection. Then, new healthy scab would form in front of the new skin, i.e. ever closer to the centre of the tail tip, increasingly in the form of only a moist brown film, with a reducing amount of discharged debris caught within it, as the infection reduced in severity 16, 18, 19, 20, 21, 23, 25.

The infection in the tail gradually subsided and, via autolytic debridement, the tissue was cleared of the infectious debris, including a foreign body in the shape of a big knot, pertaining to the stitch, stuck deep in the soft tissue and brought forward to be expelled via the wound surface (pic 14, 15, 16, 17). This had on Day 9 become removable without the need for pain relief and, once removed, any remaining swelling in the tail rapidly disappeared (pic 24).

The day of the first application of MPPT, the dog still showed signs of pain (panting) but the pain subsided into the late evening, and the dog had a quiet night's sleep. 24 hours after the first application, the dog was reported as "much more relaxed" and "not bothered about her tail at all". She was back to eating normally and her faeces was "back to normal". Neither the dog herself nor the two other dogs in the family showed interest in the tail. It was still sore if she sat on it, though. She soon learned to associate the improvement with the MPPT as she on the second day of treatment was already reported to be "just standing still and letting her owner do the washing and treating". After 1.5 weeks of treatment the dog was reported to be "very alert and just wanting to go out into the woods" with the owner concerned that she "did not stop wagging" whenever she was out! This did however not influence the tail adversely.

Despite the soft tissue granulating quickly to cover and protect the end of the cut tail bone (pic 15, 16, 18, 19) and enabling new skin to move in from all sides to protect it (pic 19, 20, 21), a distinct circular gorge formation, looking like a "moat" (pic 15, 16, 18) remained in the location of the presumed circumference of the cut tailbone for an unusual length of time in an otherwise well granulating and epithelializing wound (pic 19, 20, 21, 22). Gorge formation in connection with an underlying bone is a typical sign of infection in the bone (osteomyelitis) as the gorge constitutes the path through which the debris, that is continuously created by the infection inside the bone, travels to the surface of the body to be disposed of. This debris is infective and has a "corrosive" (necrotising) impact on the

soft tissue causing soft tissue breakdown if not disposed of properly as directed and effected by the immune system. The fact that the distinct circular gorge was not disappearing despite the rapid progress seen in all the soft tissue, was therefore a concern.

This clinical picture of the wound concurred with the medical history. The second amputation, unlike the first one, had been performed cutting perpendicularly through a vertebra and suturing the soft tissue directly up against this cut surface to cover it. As the soft tissue was likely to hold a dysbiotic microbiome with a large infective potential, this meant, that the very delicate, cut periosteum, cut internal bone structure of canaliculi and osteocytes as well as the cut bone marrow were lying unprotected up against infected soft tissue providing the infection unhindered access to the internal bone tissue.

The infective agent was clearly antibiotic resistant as the dog had been on 3 courses of antibiotics, only for the infection to return stronger after each course. Antimicrobial resistance and bacterial virulence are linked (Bengoechea and Sa Pessoa 2019) and a typical virulence factor is abscess formation. Indeed, on Day 20, (pic 22) a relatively important abscess evacuated its content though the gorge onto the surface. Abscesses typically hold concentrations of bacterial toxins and can be different colours depending on the microbe. The toxins in this relatively big abscess were red-pigmented (pic 22). (Please note the pronounced difference in the 48 hour interval between Day 19 and Day 21 (pic 21, 22, 23).

Getting rid of the accumulated toxins via these evacuations seemed to increase the dog's wellbeing considerably as this evacuation coincided with reports stating that "the dog's coat has changed back to its usual silky and soft" condition and that the dog was "totally alert" and "really back to being her own self now" – her own self being a typical active, young springer enjoying lots of unrestrained of exercise.

This evacuation seemed to be the final of relatively large soft tissue abscesses as it prompted the healing to progress beyond the gorge filling in with granulation and new epithelium (pic 25) and practically close the wound (pic 26). The removal of the osteomyelitis as well as the removal of all abscesses disseminated in the soft tissue is essential to achieve *stable* closure. The present antibiotic resistant infection was characterised by many small abscesses distributed throughout the soft tissue. These went on to be evacuated and dealt with over the following weeks and are visible in the pictures as dark specs in tiny crusts on top of the new skin remaining for a few days and then shedding off (pic 25, 27). Every time, they would leave healthier, more functional new tissue and skin behind covering the stump, including increased pigmentation and hair follicles (pic 26, 27).

The only concern was the osteomyelitis that the wound was still dealing with. On Day 40, when the wound was closed from a soft tissue perspective, the wound evacuated a collection of bacterial toxins coming from the underlying bone through a gorge structure resembling the already familiar "moat" directly overlying the amputated bone, (pic 28). Bone infection is known to be very hard to deal with and tends to become chronic. As MPPT works to support and strengthen the immune system, the dog's immune response was able to deal with it over the following months. The visible signs of these proceedings were the expulsions of toxins through the new skin directly overlying the amputated end of the bone where the infection had encountered unhindered access. A sign of continued improvement was the fact that the expulsions would continue and that every expulsion would be less intense, i.e. delivering a smaller amount of toxins and causing less harm on its way out, and would be followed by an immediate and uncomplicated restoration of the skin with an ever-increasing amount of pigmentation and hair follicles. (pic 28 to 33 & 34 to 36 & 37+39).

The dog was leading a normal life. 2.5 months after the MPPT treatment was started, she was holidaying on the beach and bathing in the ocean. To aid the immune system in its fight against the osteomyelitis, and to ensure that the skin would always close readily following the toxin expulsions, the MPPT was kept up for a time with a diminutive amount of powder applied to the few square mm of the upmost tip directly overlying the bone, gradually weaning the MPPT off altogether by increasing the intervals. The infection in the bone finally gave way and the last few millimetres of the new skin covering the tip of the tail settled

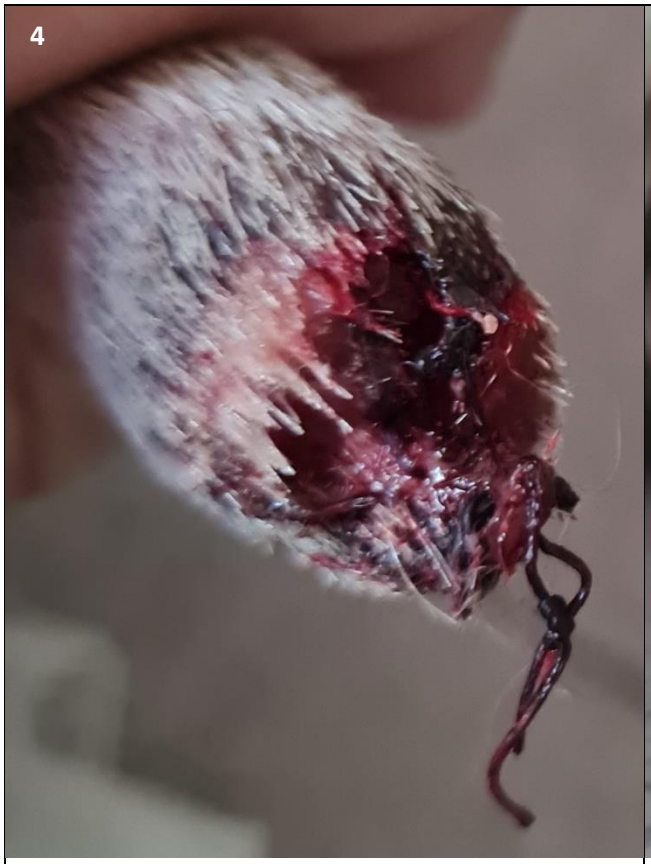
with pigmentation, hair growth and negligible scar tissue (pic 40), which continued to mature and remodel into increasingly normal skin over the following months (pic 41, 42).

Day minus-28
8 days before first amputation
4 weeks before start MPPT

Day minus-26
6 days before first amputation
4 weeks before first MPPT

Day minus-26
6 days before first amputation
4 weeks before first MPPT

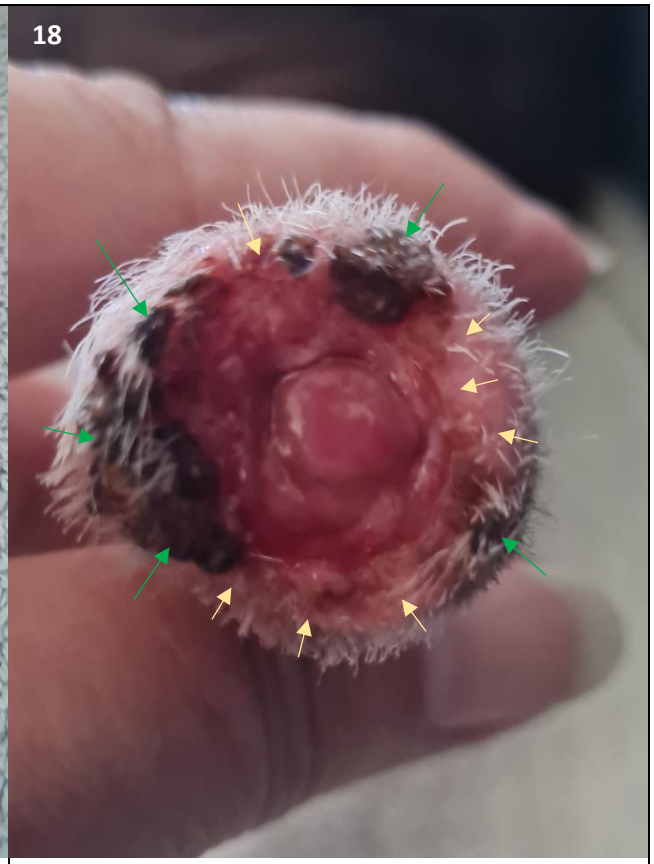
Seen by vet. Scheduled for amputation 6 days later.

		
Day minus-11 **9 days after first amputation** **1 day before second amputation** **1½ weeks before first MPPT** The entire wound bed is necrotic. Loose stitches are hanging in the last bits of the soft tissue in which they were attached after this has disappeared. The necrotic processes continue to advance in cranial direction as the infection is spreading up the tail.	**Day minus-5** **5 days after second amputation** **1 week before first MPPT** Necrosis along entire suture line. The expected fusion of the two incision lines is not evident. The tail is swollen and inflamed.	**Day minus-2** **8 days after second amputation** **2 days before first MPPT** The sides have fully separated. All tissue at the end of the tail is non-viable and the necrotising infection is gaining strength. Swelling and inflammation has reached far up the tail cranially.

7	8	9
		
Day minus-1 **9 days after second amputation** **1 day before first MPPT**	**Day 0 A** **Before removing loose necrotic lump only attached by a stitch** **10 days after second amputation**	**Day 0 B** **After liberating the tail from the necrotic lump and washing** **Just before first MPPT**
The bottom half of the tail tip in the picture is dark and non-viable. The upper half is necrotic, as well, with clear multi-tunnel formation presumably for the escape of gases from the non-aerobic infection.	The tail is swollen, warm and exuding. the skin is thinning and retreating. The infection is moving up the tail in cranial direction, visible as inflammation and dark necrotic patches in the skin for at least 6 cm. The tip of the tail is red but not granulating and in the centre is a deep, distinct, round tunnel of 2 - 3mm diameter leading directly to the tail bone. *White arrow*: The stitch keeping the lump attached.	*White arrow*: The cut stitch left in the tissue.

10 	11 	12 
Day 0 C **first MPPT application** The wound is covered with a non-broken layer of MPPT, including all inflamed or otherwise affected skin.	**Day 1** **After 12 hours with MPPT** Granulation. All the edges are covered with the distinct brown film that protects epithelialisation. The wound bed is covered with granulation germ cells. These are pale and transparent until angiogenesis occurs which gives them their typical pink/red appearance. *White arrow*: The stitch left in the tissue.	**Day 2** The tail is less swollen but some swelling remains and the inflammation remains visible as redness of the skin.

13 	14 	15 
Day 3 Inflammation is gone. Very slight swelling remains, mainly at the distal end.	**Day 4** The stitch is being loosened and pushed out as a foreign body by way of autolytic debridement. A healthy crust has formed on the edges of the weakened skin. This is a pronounced form of the typical brown film that usually protects ongoing epithelialisation underneath it. This scab falls off when the new skin underneath it is sufficiently mature and ready to let go of its protection.	**Day 5** The typical gorge formation in the shape of a circular “moat” is visible in the centre. The centre of the bone is covered by soft tissue, which protects it. More suture material is visible indicating that it is getting closer to the wound surface.

16 	17 	18 
Day 9 The stitch had been well loosened and rejected by the tissue. It was now evident that a buried bulky knot was retaining it in the tissue. This was easily removed without the need for local anaesthesia or analgesia.	**Day 9** All inflammation is gone. The distal end seems slightly swollen still due to the stich and the osteomyelitis, but the picture is deceiving due to the relatively long hair on the non-shaved, delicate area of the amputation site. *Orange arrows*: pigmentation is restored. *White arrow*: The stitch left in the tissue	**Day 10** The stitch had been removed the previous afternoon. The typical gorge formation in the shape of a “moat” remains visible in the centre. *Green arrows*: Protective scab and brown epithelialisation film remaining in place. *Cream arrows*: The protective scab has fallen off in some areas of the wound edges exposing new epithelium underneath mature enough to let go of its protection.

Day 11

The soft tissue is building up via granulation. The skin is rapidly moving in from all sides. The central gorge formation remains as an indicator of osteomyelitis.

Day 14

The skin is now covering everything except the centre demarcated by the gorges. The soft tissue coverage of the bone is increasing in thickness.

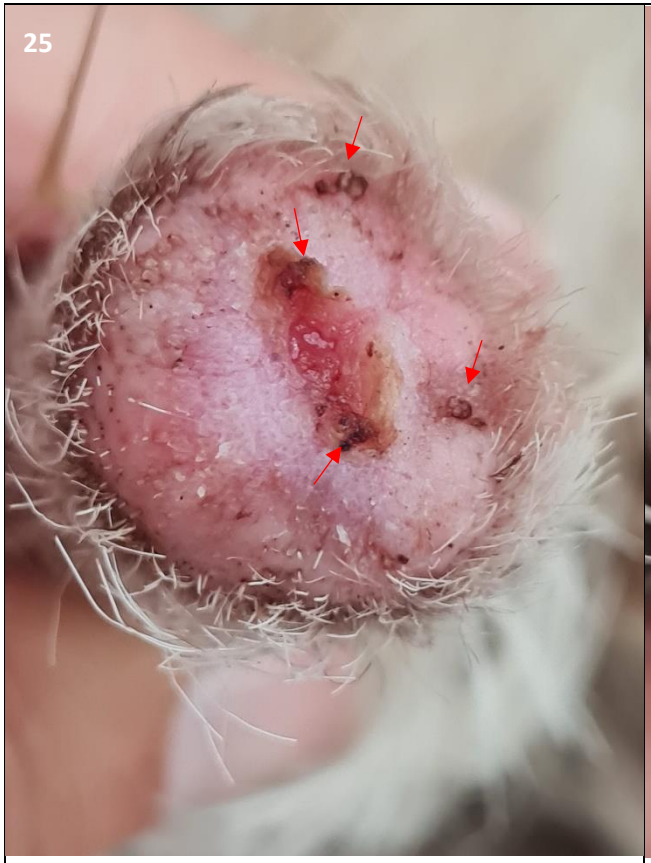
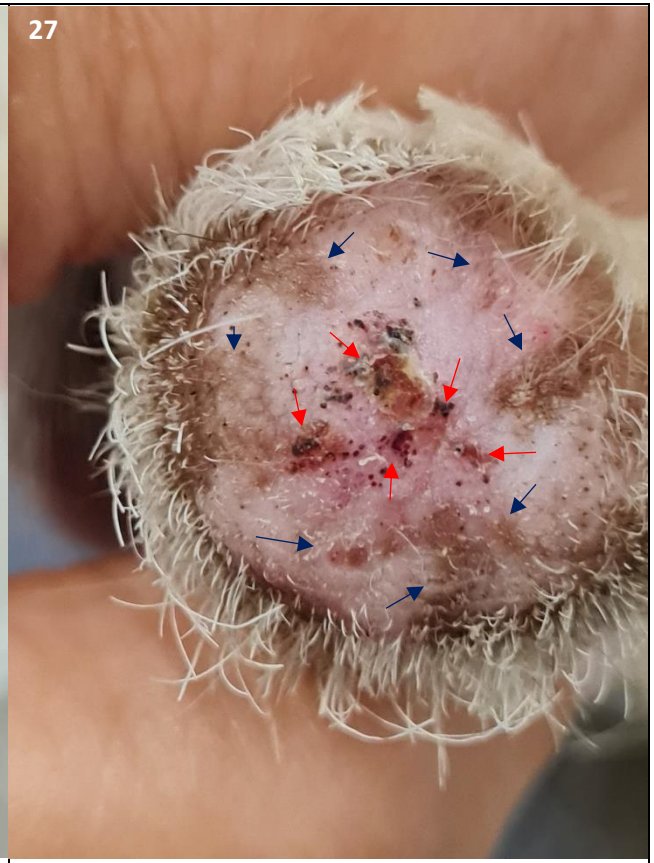
Day 19

Everything is improving. Just waiting for the osteomyelitis to clear so that the gorges can close.

A bone infection (osteomyelitis) continuously creates debris that the body needs to dispose of. The gorges are the channels through which the infective debris travels from the bone to the surface.

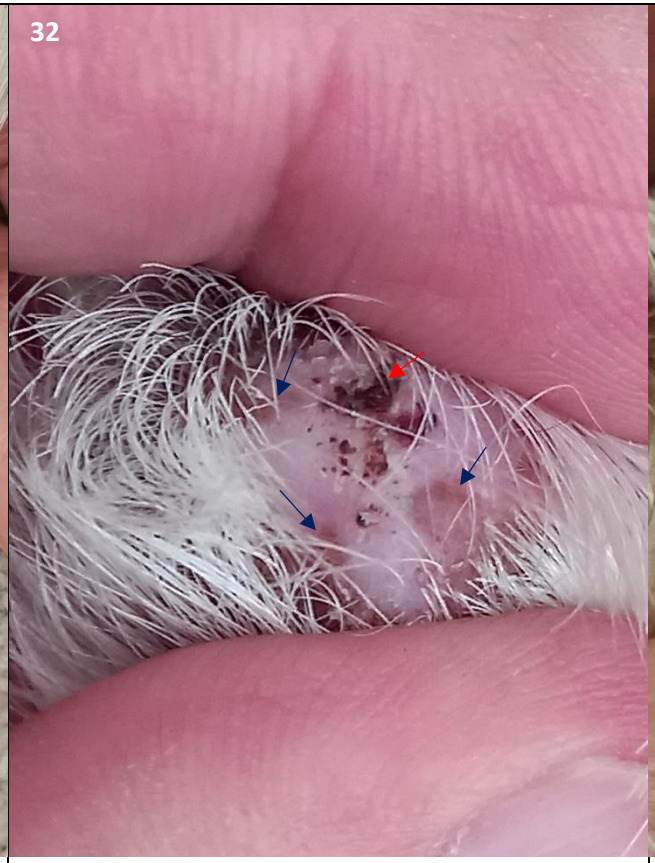
In infections characterised by abscesses disseminated in the soft tissue, the immune system will typically use such already established canals to also direct the debris evacuated from the abscesses.

 22	 23	 24
Day 20 Red bacterial toxins and debris evacuated from a hidden bacterial abscess are disposed of through the gorges onto the surface. This phenomenon is sometimes mistaken for bleeding but the toxins are characteristically even “redder” and they do not change colour upon contact with oxygen. The picture was captured just when an expulsion of toxins was taking place. Presumably the abscess was the cause of the last remaining bit of apparent swelling at the distal end as this now fully disappeared (see pic 24).	**Day 21** The gorges are less distinct. Also, the central area is closing further. The brown epithelialisation protecting film is visible around the entire wound edges.	**Day 22** No swelling remaining even at the distal tip. Full pigmentation and good hair growth.

		
Day 24	**Day 30**	**Day 35** **One month** **Seemingly closed**
Centre filling with healthy granulation. Strong epithelialisation. The infective, toxic content of small abscesses dissiminated in the soft tissue and stemming from the osteomyelitis is being transported to the surface and disposed of on the surface. They form tiny scabs for a few days before falling off. *Red arrows*: Tiny toxin containing scabs. The two most central ones caught in the brown epithelialisation film is presumably from the osteomyelitis, whereas the two further away from	The wound is practically closed. 1x2 mm granulation tissue in the centre remains to be covered with epithelium. *Dark blue arrows*: Pigmentation is moving into the newly generated epithelial cells as they mature, ensuring good function of the skin.	All soft tissue is now restored and the wound as such closed. Toxin containing abscesses disseminated in the soft tissue have been depleted and the soft tissue restored and epithelialized. Toxin evacuations are now limited to the central area directly overlying the bone. *Red arrows*: Tiny toxin containing scabs. *Dark blue arrows*: Pigmentation demonstrating healthy skin function.

the underlying bone presumably are from abscess evacuations.		
		
Day 40	**Day 44**	**Day 48**
A sudden outflux of infective debris from the bone infection forces its way through the central epithelium which is covering the area that was formerly a circular gorge.	The central part is restoring the damaged centre. The entirety of the skin seems healthier.	A scab is covering the central part protecting the final reparations to the skin taking place underneath. The skin surrounding the scab shows no signs of further abscess evacuations.

Only once the osteomyelitis is fully removed can the overlying wound close definitively. Until such time, the debris created in the bone must find a way out through the skin. The immune system will keep the soft tissue covering the infected bone free of infection and the canal between the bone and the body surface as small and narrow as possible, whilst waiting for the immune system to combat the infection in the structures of the bone. As the bone infection reduces the expulsions of debris wane and become less and further apart.

31	32	33
		
Day 60 **2 months** Steady hair regrowth offers good protection.	**Day 62** **2 months** The new skin, generated to cover the amputated bone and the restored soft tissue around the bone, has hair follicles and pigmentation indicating its full functionality. *Red arrow*: Tiny toxin containing scab of approximately 1x2mm protecting the centre just overlying the bone. *Dark blue arrows*: Pigmentation entering the new skin as it matures. Hair is already growing well.	**Day 75** **2½ months** **Closed** Scab has fallen off and reveals perfectly healthy and matured skin underneath. *Red arrow*: Lower: Single 0.5x0.5mm spec of toxin making its way through the new epithelium. Upper: cluster of approximately 7 specs of such toxins. Once all through the skin, they will fall off unnoticed. *Dark blue arrows*: Epithelial maturation with pigmentation and hair growth.

34 	35 	36 
Day 82 **2 2/3 months** Outflux of debris from the bone infection, mainly red-pigmented toxins precisely overlying the bone amputation site. Reduced in intensity compared to last expulsion (Day 40). Pigment and hair still progressing.	**Day 90** **3 months** Toxins have reached the uppermost layer of the epidermis and are falling off. The skin underneath is new, healthy and maturing. *Red arrow*: Remaining cluster of specs of toxins.	**Day 108** **3½ months** Toxins have cleared – only one spec left. The Epithelium is perfectly healthy and functional with no signs of settling as scar tissue. Only 1x1mm in the absolute centre directly overlying the amputation (*white arrow*) seems very slightly rougher than everything else whilst awaiting epithelial repair. *Red arrow*: Two fused specs of toxin.

Day 152

5 months

Outflux of infectious debris from the bone, mainly red-pigmented toxins, precisely overlying the bone amputation site. Again considerably reduced in intensity compared to last expulsion (Day 82).

Red arrow: Small cluster of specs of toxins.

Day 164

5 1/3 months

Well protected tail tip with hair regrowth fully restored.

Day 173

5½ months

As the novel skin matures, the pigmentation moves in ever further.

Red arrow: The latest cluster of specs are in the final process of shedding off. The characteristic shape of this repair remains that of a round “moat” and, again, it precisely overlies the amputation site of the bone, indicating that the toxins originate in the bone structure.

White arrow: A typical cap of protection under which a small repair job is taking place. It is most likely that the new, still maturing epithelium has been slightly damaged, through normal activities in a dog’s life.

40 	41 	42 
Day 199 **6½ months** **Follow-up** Pigmentation has fused. Hair follicles continue to appear in the new skin. The fully functional new skin will continue to mature and remodel over the following months and year(s).	**Day 313** **10 1/3 months** **Follow-up** The stump is covered with healthy skin. Only the precise outline of the cut tail bone lacks pigmentation. Remodelling will continue for a long time, further strengthening the resiliance of the area.	**Day 313** **10 1/3 months** **Follow-up** The scar is minimal and well buried in and protected by lavish hair growth.