ZOO ANIMALS

Fatal bacterial septicaemia after immobilisation of a captive brown bear (Ursus arctos)

Elfi Katrin Schlohsarczyk, Nadine Schmidt, Ellen Prenger-Berninghoff, Leonie Franziska Herkommer, Manfred Henrich

SUMMARY
A four-year-old female brown bear (Ursus arctos) was immobilised via blowpipe targeting the right hind limb to perform a chemical contraception. Few days after anaesthesia, the animal developed lameness and a reduced general condition, and died 1 week after immobilisation when alopecia and swelling of the injection area were observed for the first time. Besides a severe azotaemia, alopecia and reddening of the cranio medial skin, subcutis and skeletal muscles of the entire right hind limb showed a phlegmon, oedema and haemorrhage. Additional widespread haemorrhages were found. Histology revealed a supplicative and necrohaemorrhagic myositis of the affected limb, a supplicative iliofemoral lymphadenitis, blood resorption in the mesenteric lymph nodes and haemorrhage in the adrenal gland cortex. Microbiological investigation revealed high amounts of Escherichia coli, Streptococcus dysgalactiae and Clostridium septicum in skin, musculature, heart, lung, liver and spleen, indicating septicaemia, which led to death due to toxae mic shock.

BACKGROUND
This report illustrates the occurrence of a suppurative and necrohaemorrhagic myositis, as well as a severe phlegmon of the right hind limb of a captive brown bear (Ursus arctos), accompanied by generalised haemorrhage due to secondary wound infection by Escherichia coli, Streptococcus dysgalactiae and Clostridium septicum, which occurred at the injection site after immobilisation resulting in septicaemia and death. We describe gross and histological findings as well as the microbiological results of the examined affected tissues and organs.

This is the first description of a fatal septicaemia due to simultaneous secondary infection of the injection site with S dysgalactiae, E coli and C septicum in a captive brown bear after immobilisation. So far, there have been only a few cases of clostridial necrohaemorrhagic myositis in wild Ursidae. Recently, C septicum was reported in a single wild brown bear after muscle damage, similar to other clostridial infections, such as C sordellii, C bifermants, C chauvoei and C novyi. Evidence of C septicum in a wild black bear (Ursus americanus) was associated with the immobilisation, just as in the present case in which C septicum is reported for the first time in a captive brown bear. The aim of this report was to make veterinarians who work with zoo and wildlife animals aware of the fact that secondary bacterial infections after immobilisation can lead to death of Ursidae, with death occurring as early as 48 hours after infection with C septicum.

CASE PRESENTATION
A four-year-old female brown bear, kept in a wildlife park, was supposed to be applied a drug for contraception. Therefore, the animal was immobilised by blowpipe with 3 ml of the Hellabrunner mixture (500 mg xylazine [1.5 mg/kg intramuscular Xylased, Bioveta] in 4 ml of 10 per cent ketamine [1.2 ml/kg intramuscular ketamine 10 per cent PRO INJ, Alfasan]). The Hellabrunner mixture was injected intramuscularly in the right thigh by blowpipe. Due to problems with preparing, the drug for contraception could not be used. The bear was under anaesthesia for 6 hours and woke up uneventfully but slowly. The animal appeared physically fit on the next day. Three days after immobilisation, the bear’s general condition was slightly reduced, accompanied by mild lameness and mild tachypnoea. No further clinical findings were observed, so that a further clinical investigation, which would only have been possible after a renewed immobilisation with the resulting anaesthetic risks was waived.

To relieve the pain, flunixin (1 mg/kg intramuscular Flunixin Injection, Norbrook) was applied via a blowpipe in the same area as the Hellabrunner mixture, as the animal lay on its left side in its enclosure. There was then an improvement in lameness. Licking of the injection site by the animal itself and by its mother was observed 3 days after the pain treatment. Except for a reduced general condition, no further clinical signs were noted then. Unexpectedly, the bear was found dead in its enclosure on the next day, and alopecia as well as swelling of the right hind limb were noted. The animal was submitted for necropsy.

INVESTIGATIONS
The female brown bear (190 kg) was in good body condition. However, body and tissues were affected by marked postmortem changes. The right hind limb was swollen with a 35 × 15 cm alopecic and dark red discoloured area on the cranio medial thigh (figure 1). The entire subcutis and skeletal muscles of the right hind limbs, including the paw, showed a severe diffuse phlegmon, an oedema and extensive haemorrhages (figure 2). The exact location of the injection site was not identifiable. Severe multifocal to coalescing haemorrhages were
Figure 1  Right hind limb of a female brown bear (Ursus arctos). Alopecia and dark red discolouration of the skin are shown.
Figure 2  Right hind limb of a female brown bear (*Ursus arctos*). Severe phlegmon, oedema and haemorrhage of the skeletal musculature are shown.
found subpleurally, subepicardially and in the cranial aspect of the mediastinum. The lung also showed multifocal to coalescing alveolar haemorrhages, as well as an acute diffuse severe alveolar oedema. The urea concentration in the aqueous humour was 40 mmol/l (reference value: up to 29.6 mmol/l).  

The macroscopic findings were confirmed histologically. Additionally, a suppurrative and necrotising myositis of the right hind limb, a suppurrative iliofemoral lymphadenitis, blood resorption in the mesenteric lymph nodes and a severe haemorrhage in the adrenal gland cortex were detected. Specimens of affected skin and musculature, as well as heart, lung, spleen and liver, were submitted for microbiological investigation.

Organ material was flamed and the fresh cut was streaked onto a blood agar plate with 5 per cent sheep blood (both Thermo Scientific Oxoid, Fisher Scientific), water-blue metachrome-yellow lactose agar (acc. to Gassner, E., Merck) and brain heart infusion (BHI) agar (Thermo Scientific Oxoid, Fisher Scientific). Subsequently, plates were incubated for 24 and 48 hours under aerobic or (BHI) CO₂-enriched (10 per cent) conditions. Additionally, Schaedler agar (Becton Dickinson) was used for anaerobic growth examination in an anaerobic jar system (Thermo Scientific Oxoid, AnaeroGen; Fisher Scientific) after 72 hours incubation at 37°C. Morphologically diverse colonies were counted semiquantitatively and were subcultivated. Pure cultures were identified using Matrix-Assisted Laser Desorption-Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS) (Biotype V3.3.1.0, Bruker Daltonics) and the DB 5989 database. In multiple organs, the microbiological investigation revealed bacteria with pathogenic potential in animals (table 1). C septicum was identified with a score value of 2.057 as best value.

### Learning points

- This is the first description of a fatal septicemia due to simultaneous secondary infection of the injection site with Streptococcus dysgalactiae, Escherichia coli and Clostridium septicum in a captive brown bear after immobilisation.
- To date, there have been a few reports of clostridial necrohaemorrhagic myositis following previous muscle damage, including tissue perforation via a blowpipe, for wild Ursidae. Clostridium species must be regarded as life-threatening pathogens after secondary wound infection and can lead to death within a few days. C septicum is a ubiquitous soil bacterium that can cause death within 48 hours.
- Due to the limited possibilities of the clinical investigation of wild animals, several cameras installed in the enclosure can be an additional aid in observing those animals in which only minimal clinical signs have been detected. This can enable earlier and likely life-saving decisions about the need for immobilisation for further examinations and therapies.

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**Table 1 Microbiological results of skin and skeletal musculature of the right hind limb and organ samples from a female brown bear (Ursus arctos)**

<table>
<thead>
<tr>
<th>Localisation</th>
<th>Clostridium septicum</th>
<th>Escherichia coli</th>
<th>Streptococcus dysgalactiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>++ +*</td>
<td>++†</td>
<td>++</td>
</tr>
<tr>
<td>Skeletal musculature</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Heart</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Lung</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Spleen</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Liver</td>
<td>++</td>
<td>++†</td>
<td>(+)+j§</td>
</tr>
</tbody>
</table>

*High bacterial content (>200 cfu/agar plate).
†Moderate bacterial content (50–200 cfu/agar plate).
§Single colonies.
ch, colony-forming unit.

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**OUTCOME AND FOLLOW-UP**

This case report illustrates the occurrence of a suppurrative and necrohaemorrhagic myositis as well as a severe phlegmon of the right hind limb of a brown bear, accompanied by generalised haemorrhage. The microbiological investigation of affected skin and skeletal muscle samples revealed high to moderate amounts of C septicum, E coli and S dysgalactiae, which were also detected in heart, lung, liver and spleen in a variable burden. These findings indicate a septicaemia of these bacteria originating from the inflamed injection site of the right hind limb most likely due to secondary bacterial contamination.

The inoculation of the bacteria may have been caused by the bear and its mother licking the injection site, but a smear infection with contaminated soil may also be possible. Another way of bacterial uptake could have been contaminated reusable blowpipe syringes or drugs. However, this is almost impossible because the dart syringes were carefully cleaned and sterilised after each use and no animal that had been immobilised after the bear showed clinical signs or died.

The alopecia of the right hind limb is most likely the result of the licking first observed the day before the animal died.

Death due to septic shock leading to multiorgan dysfunction often occurs within 48 hours after infection with C septicum. In the presented case, the bear died 7 days after immobilisation. It would be possible that the primary injection site infection was from inoculation of E coli and S dysgalactiae. This, in turn, led to the suppurrative myositis and phlegmon. The resulting pain then led to an initially reduced general condition and lameness. The subsequent further reduction in general condition on the sixth day after immobilisation, as well as the sudden death, could have been promoted by the additional infection with C septicum. This probably manifested in the necrohaemorrhagic character of the myositis and likely appeared few hours to a few days before the bear died. A reported case of a cow infected with C septicum that died within a few days after calving and becoming lame could support this assumption.

However, it is likely that all three bacterial species detected (C septicum, S dysgalactiae and E coli) were significantly involved in the development of the septic shock that led to the death of the brown bear.

The lipopolysaccharide (LPS) is located in the outer membrane of Gram-negative bacteria, with lipid A having the endotoxin activity of the LPS. In relation to the high levels of E coli in the skin and skeletal musculature, it is possible that high levels of endotoxin led to the induction of excessive cytokine levels, resulting in septic shock with disseminated intravascular coagulopathy (DIC) and widespread haemorrhages after increased capillary leakage.

*S dysgalactiae* is divided in two subspecies based on phenotypical and genotypical examinations: *S dysgalactiae* subspecies *dysgalactiae* causes infection of animals (especially bovine mastitis), and *S. dysgalactiae* subspecies *equisimilis* is often isolated from humans.

Cheníer et al describe a severe cellulitis in a Brown Swiss cow due to infection with *S dysgalactiae* subspecies *dysgalactiae*, but the path of bacterial uptake remained unclear. The necropsy revealed scattered necrohaemorrhagic foci in the muscles of the hind limbs and coxofemoral region that were not associated with the overlying cellulitis. In addition, the subcutaneous tissue showed marked damage, including tissue perforation via a blowpipe, for wild Ursidae. *Clostridium* species must be regarded as life-threatening pathogens after secondary wound infection and can lead to death within a few days. *C. septicum* is a ubiquitous soil bacterium that can cause death within 48 hours.
congestion, oedema, haemorrhage and moderate fibrinopurpurative inflammation. All of the findings supported streptococcal toxic shock-like syndrome (TSS).  

The second subspecies of *S. dysgalactiae*, *S. dysgalactiae* subspecies *equisimilis*, can cause cellulitis and TSS, in addition to other inflammatory lesions in humans.  

This agent may also cause diseases in animals. One case report describes a severe disseminated suppurrative meningoencephalomyelitis in a Yorkshire pig due to *S. dysgalactiae* subspecies *equisimilis*.  

It causes cytolytic activators release toxins, whereby the alpha toxin, an exotoxin, is the main virulence factor. The alpha toxin consists of two subunits, an A subunit, a DNA gyrase inhibitor, and an B subunit, an adenylate kinase that inhibits adenylate kinase of the host cell.  

The damage of *C. perfringens* subspecies *equisimilis* was also isolated from nasal swab samples collected from horses with a history of respiratory diseases.  

*S. dysgalactiae* subspecies *equisimilis* belongs to the most common pathogenic streptococcal species in the dog and may cause septicaemia as well as life-threatening localised infections in the skin and lung.  

It was also observed that *S. dysgalactiae* subspecies *equisimilis* could be isolated from several healthy dogs with frequent contact with horses.  

Streptococci are normal flora components of the skin and gastrointestinal tract in dogs. Thus, the isolation must be interpreted with consideration of clinical and pathological findings.  

So far, *S. dysgalactiae* subspecies *equisimilis* has not been described as a normal component of the skin flora in Ursidae.  

Classical TSS is caused by superantigens of *Staphylococcus aureus* and *S. pyogenes*. The effects are similar to those caused by septic shock. Superantigens are a form of exotoxins from Gram-positive bacteria that cause TSS by stimulating up to 20 per cent of T lymphocytes, resulting in massive T lymphocyte proliferation and cytokine release. Both *S. dysgalactiae* subspecies *dysgalactiae* and *S. dysgalactiae* subspecies *equisimilis* can have genes that encode superantigens.  

Chérier et al, on the other hand, could not detect superantigen genes so that it was assumed that TSS could have been caused by other virulence factors of *S. dysgalactiae*.  

In our case, the subspecies of *S. dysgalactiae* had not been determined, and no further investigations for the presence of superantigens were carried out, so that the relevance of superantigens or other virulence factors of *S. dysgalactiae* cannot be excluded.  

*C. perfringens* is a ubiquitous bacterium forming spores within soil. It is a large Gram-positive strict anaerobic bacillus, known to cause the so called ‘malignant oedema’ (or ‘gas gangrene’). Disease may occur if spores enter wounds as secondary wound infection as in this report. Gas gangrene following this pathomechanism often occurs in horses. Gas gangrene due to penetrating contaminated objects which carry spores into the wound is often reported in ruminants. Gas formation is a common feature but was absent in this case. Clinical signs are heating, swelling, and pain of affected musculature as well as fever, depression, dehydration and anorexia. The injury causes an anaerobic microenvironment with lowering of the redox potential that leads to germination of spores. Subsequently, the vegetative bacteria release toxins, whereby the alpha toxin, an exotoxin, is the major lethal factor produced by *C. perfringens*. It causes cytolytic activities due to pore formation, resulting in haemolysis and destruction of the endothelial cells of the microvasculature.  

The damage of blood vessels leads to extensive haemorrhage (alveolar, subpleural, subepicardial, mediastinal, in the mesenteric lymph nodes, as well as in the adrenal gland cortex), what can be also promoted by the pathogenic mechanisms of *E. coli* and *S. dysgalactiae*. Tissue oedema and necrosis of musculature as well as a decreased blood flow to the site of infection results in ischaemia that favours the further survival of *C. perfringens*.  

In this case, the generalised haemorrhage is also the result of an increased consumption of coagulation factors and platelets in the course of a DIC caused by *S. dysgalactiae*, *E. coli* and *C. perfringens*. The DIC is provoked by the complement cascade, activated by bacterial components. Additionally, a widespread vascular leakage, induced by inflammatory mediators, leads to haemorrhage and the observed tissue oedema in the course of septicaemia. In this context, the severe alveolar oedema can also be interpreted as a consequence of elevated vascular permeability, as well as a result of agony.  

Furthermore, the alpha toxin of *C. perfringens* inhibits polymorphonuclear leucocyte infiltration of infected lesions. So, the lack of inflammatory cells in infected tissues is typical of clostridial myonecrosis. This indicates that the accompanied phelegmon and the suppurrative inflammation of the skeletal muscles are most likely provoked by the infection with *S. dysgalactiae* and *E. coli*.  

The right hind limb is drained by the iliofemoral lymph nodes what consequently caused the suppurrative lymphadenitis in this case.  

The severe azotaemia is most likely a prerenal form as a consequence of reduced water intake due to the decreased mobility of the animal in the course of the disease.  

No morphological changes were found being responsible for the long hypnogenic state after immobilisation. One year before the animal’s death, the Hellabrunner mixture had been applied to this bear without any complications. Furthermore, the animal had never developed any case of illness.  

**DISCUSSION**

In a recent study illustrating the causes of deaths of free-ranging brown bears in Spain, five brown bears showed gangrenous myositis, widespread haemorrhages and oedema as a consequence of wire snare hunting, traumatic lesions or fighting. *C. sordellii* was isolated as the etiological agent in four bears. In addition, *C. perfringens* and *C. bifermentans* were detected in two of these four animals.  

In one bear suffering from gangrenous myositis, *Clostridium* species could not be isolated.  

A 1980 case report describes clostridial myonecrosis in a wild black bear (*U. americanus*) that had been captured and immobilised in Minnesota and died 3 days after immobilisation. The lesions that were similar to those as in this report were tested positive for *C. perfringens*, *C. chauvoei* and *C. noxia*. The exact path of bacterial uptake was unclear, but inoculation through the injection needles used for immobilisation or through the drug itself were considered as probable pathways.  

In addition, necrohaemorrhagic lesions due to *C. perfringens* have also been reported in several animals, including one lynx and one gorilla, most likely after secondary infection of traumatic wounds. One study describes necrohaemorrhagic lesions in the skin and muscles of broiler chickens (gangrenous dermatitis) due to an outbreak of *C. perfringens* and *C. perfringens* infection. There is also a case report about myonecrosis due to *C. perfringens* infection in a dog with myiasis. In addition, gas gangrene caused by *C. perfringens* is reported in goats, considering endogenous infection due to pregnancy-induced immunosuppression due to the absence of skin lesions.  

Similar lesions occur in blackleg disease of ruminants after oral infection with *C. chauvoei*. In contrast to *C. perfringens*, *C. chauvoei* spores reach the muscles through hematogenous spread from the intestine. Blackleg often follows traumatic injury to muscle that creates an anaerobic environment stimulating germination of spores. Subsequently, the vegetative bacteria release toxins, whereby the alpha toxin, an exotoxin, is the major lethal factor produced by *C. chauvoei*. It causes cytolytic activities due to pore formation, resulting in haemolysis and destruction of the endothelial cells of the microvasculature.  

The damage of blood vessels leads to extensive haemorrhage (alveolar, subpleural, subepicardial, mediastinal, in the mesenteric lymph nodes, as well as in the adrenal gland cortex), what can be also promoted by the pathogenic mechanisms of *E. coli* and *S. dysgalactiae*. Tissue oedema and necrosis of musculature as well as a decreased blood flow to the site of infection results in ischaemia that favours the further survival of *C. chauvoei*.  

In this case, the generalised haemorrhage is also the result of an increased consumption of coagulation factors and platelets in the course of a DIC caused by *S. dysgalactiae*, *E. coli* and *C. perfringens*. The DIC is provoked by the complement cascade, activated by bacterial components. Additionally, a widespread vascular leakage, induced by inflammatory mediators, leads to haemorrhage and the observed tissue oedema in the course of septicaemia. In this context, the severe alveolar oedema can also be interpreted as a consequence of elevated vascular permeability, as well as a result of agony.  

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The severe azotaemia is most likely a prerenal form as a consequence of reduced water intake due to the decreased mobility of the animal in the course of the disease.  

No morphological changes were found being responsible for the long hypnogenic state after immobilisation. One year before the animal’s death, the Hellabrunner mixture had been applied to this bear without any complications. Furthermore, the animal had never developed any case of illness.
addition, the use of antibiotics would have posed several difficulties. Oral administration of drugs is unreliable in wildlife because it is uncertain whether they are fully absorbed. Application via a blow-pipe, on the other hand, required a drug that had such a small injection volume that it could be used with one or just a few dart syringes. Approved antibiotics for this use are very limited to non-existent. Furthermore, anaesthesia with the subsequent manual application of antibiotics would have been another option. However, this would have to be repeated for a few days, which would have brought the risk of anaesthesia again with it. Because of the bear’s long hypnotic state after immobilisation, it was more important to consider whether further anaesthesia would have been necessary. However, the mild clinical findings did not justify immobilisation for further investigations or for the use of antibiotics. Alopecia and swelling of the right hind limb were observed for the first time on the day the bear died. The reasons for this were that these lesions were on the cranio-medial skin of the right thigh, which was difficult to see, especially when the animal was lying on its left side. For safety reasons, the clinical examination of the captive brown bear was primarily limited to the control by the veterinarian, whereby observations on behaviour, food and water consumption by the animal owners were requested.

One possibility would be to install multiple cameras in the housing. This would help to view wildlife from different perspectives, increasing the likelihood of noticing relevant clinical findings.

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