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## Health, zoonotic pathogens and parasites

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### Presence of bacteria in red deer antlers during different stages of development

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Red deer antlers grow and mineralize while being covered with velvet. Following the velvet shedding, hard antlers are revealed. There are still debates whether hard antlers are a dead bone or they survive for longer periods after velvet shedding. Since reddish liquid is frequently present on the cut surface of hard antlers and is by some authors described as blood, the aim of this study was to analyse presence of blood cells and bacteria in it.

Antlers from a red deer spiker (yearlings, i.e. in second year of life) were sampled in different stages of development: in the velvet (A1), two months after velvet shedding (A2), and three months after velvet shedding (A3). Antlers in the velvet were sampled in complete chemical immobilization. Hard antlers were removed with a fine-toothed saw approximately 5 cm above the pedicle-antler junction. After delivery to the Faculty of Veterinary Medicine, the sampled antler beams were divided into three segments (1 – proximal, 2 – middle, 3 – distal). Each segment was additionally sampled longitudinally and transversely. Before cutting, the surface of the antler and diamond separator were treated with 70% ethyl alcohol. The incision site was cooled with saline solution. Study was two folded, presence of bacteria was observed using microbiological analyses and using histological analysis. Swabs were taken from the cut surface and sent for bacteriological examination. The species were identified by the MALDI TOF method. The fragments were fixed in 10% neutral buffered formalin. The antler fragments were demineralised using Osteosens<sup>®</sup> solution (EDTA based) and subsequently washed in phosphate buffer. The fixed and demineralised fragments were embedded in paraffin and sliced to a thickness of 6 µm. The sections were stained with hematoxylin-eosin (HE) and BioGram Histo kit for differentiation between Gram-positive and Gram-negative bacteria in histology sections. Smears of liquid content from the antlers were stained with May-Grünwald Giemsa.

Red blood corpuscles and leukocytes were present only in A1. With BioGram Histo kit bacteria were visible only in the A2 and A3. Swabs from A2 were positive to *Erysipelothrix rhusiopathiae*, *Kurthia zopfii*, *Bacillus licheniformis*, *Macrococcus canis*, and *Citrobacter freundii*. Swabs from A3 were positive to *Pseudomonas flavescens*. Obligate anaerobes were not isolated from A1, A2, and A3 swabs. Study showed that antlers in the velvet contain blood without bacteria, which was expected for live tissue. After the velvet shedding, we did not find blood cells, and the analysed liquid contained bacteria, confirming the cessation of circulation during mineralization. The remaining liquid is insufficient to nourish bone cells and provides an ideal medium for bacterial growth. Most of the mentioned bacteria are present in the environment. All isolated bacteria have zoonotic potential, and infections caused by them have been confirmed in humans.