

# INTESTINAL LESIONS AND BACTERIAL COUNTINGS SUGGESTIVE OF *CLOSTRIDIUM PERFRINGENS* ENTEROTOXAEMIA AS A CAUSE OF MORTALITY IN WILD CERVIDS

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## Introduction

Since last decades, wildlife diseases have been more intensely investigated in Europe. This concerns zoonoses but also wildlife diseases transmissible to domestic animals. Some countries possess their own surveillance programmes for wildlife diseases. In Belgium, a network was created in 2001 and focused on wild cervids. This work is based on necropsies of hunter-killed wild cervids and animals found dead. The objective is to investigate the health status of wild ruminants and to determine main causes of mortality.

## Material and methods

Within 2 consecutive years, 63 free-living cervids (36 *Cervus elaphus* and 27 *Capreolus capreolus*) were found death in southern Belgium and brought to the necropsy room of the Faculty of Veterinary Medicine. A complete necropsy was conducted and focused complementary analyses were performed, depending of the macroscopic lesions observed. When lesions of acute haemorrhagic enteritis were observed, intestinal sections were sampled for bacteriological analyses. For anaerobic investigations, 100 µl of intestinal content were sampled and resuspended in 900 µl (dilution 1) of Brain Heart Infusion Broth (Oxoid). The 10-fold dilutions (2-6) were made in 900 µl of BHI. Dilutions 3-6 were inoculated with a spiral system plater (E. Jet, IUL Instruments) onto Columbia Agar plates (B-Dickinson). These plates were put overnight at 37°C under anaerobic conditions (90%N<sub>2</sub>, 5%H<sub>2</sub>, 5%CO<sub>2</sub>) in a MK3 Anaerobic Workstation (LED Techno). Identification (Api ID32A) and quantification of *C. perfringens* were subsequently realised. Three intestinal samples of cervids found dead but without enteric lesions were also analysed (negative controls).

## Results

Among the 63 wild cervids analysed :

- 17 animals displayed significant changes of the volume and/or content of mesenteric lymph nodes along with granulomatous adenitis at histopathology. Lymph nodes tested were positive by PCR for IS900 sequence of *Mycobacterium avium paratuberculosis*.
- 15 animals showed intestinal and/or pulmonary lesions compatible with polyparasitism.
- Cavitory (hemothorax & hemoperitoneum) haemorrhages, brain haemorrhages or vertebral fractures were responsible for the death of 15 cervids
- 4 animals presented diffuse or localised lesions of acute haemorrhagic enteritis (jejuno-ileal section). All these animals proved negative for *Salmonella spp.* Anaerobic bacterial counts were performed on these sections. The counting results of *C. perfringens* were between  $1.10^4$  and  $24.10^6$  CFU/ml of intestinal content. In comparison, counting results of 3 negative control (found dead but without enteric lesions) were superior to  $1.10^4$  CFU/ ml of intestinal content ( $4. 10^4$ ;  $5,5. 10^5$  &  $2,8. 10^6$ ).
- Other mortality causes are listed in “miscellaneous causes” (pasteurellosis, babesiosis, ...).

## Conclusions

This is the first survey devoted to examining the causes of mortality among wild cervids in Belgium. The 3 most frequent causes were (i) paratuberculosis, (ii) polyparasitism and (iii) traumatic lesions. Among other less frequent causes, *Clostridium perfringens* enterotoxaemia should be considered whenever segmental haemorrhagic enteritis is found. However, due to large postmortem overgrowth of the clostridial flora in these “found dead” animals, diagnosis must be considered with caution. Clostridial countings should be seen as a tool able to strengthen diagnosis rather than a decisive primary argument. Further experiments will be of interest to determine toxinotype(s) involved in wild ruminants.