

## ENTEROTOXAEMIA IN MOUFLONS (*OVIS MUSIMON*)

T. P. POPOVA<sup>1</sup>, Y. M. TZVETKOV<sup>2</sup> & TS. DIKOVA<sup>3</sup>

<sup>1</sup>Faculty of Veterinary Medicine, University of Forestry; <sup>2</sup>National Research Station of Game Preserves, Game Biology and Pathology; <sup>3</sup>National Veterinary Diagnostic and Research Institute; Sofia, Bulgaria

### Summary

Popova, T. P., Y. M. Tzvetkov & Ts. Dikova, 2007. Enterotoxaemia in mouflons (*Ovis musimon*). *Bulg. J. Vet. Med.*, 10, No 4, 267–272.

In a herd of mouflons, a lethality reaching 10% of the population, with pathomorphological signs of enterotoxaemia was observed. From all examined animals, *Clostridium perfringens* was isolated, serologically identified as type C. A carriership of *C. perfringens* type C was proved in healthy mouflons too. The principal factors, provoking the development of the disease were analyzed. The results showed a high sensitivity of mouflons to *C. perfringens*. This microorganism is probably part of the intestinal microflora in mouflons, like sheep and other animal species.

**Key words:** *Clostridium perfringens* type C, enterotoxaemia, mouflon

### INTRODUCTION

The mouflon (*Ovis ammon musimon* Schreber, 1792) is a valuable game species in our game preserves. Yet, little is known about the pathology affecting this animal species. That is why, any emergence of acute diseases and lethality creates difficulties in diagnostics and is causing losses, but at the same time, it is an occasion to study the infectious pathology in the mouflon.

Mouflons are small ruminants, physiologically close to sheep. There are publications reporting that mouflons are affected from diseases, characteristic for domestic sheep (Nattermann *et al.*, 1990; Sarrazin *et al.*, 1990; Takats, 1992; Thiry *et al.*, 1992). This is supported from our research too (Tzvetkov & Dikova, 1999).

In small ruminants, enterotoxaemia is one of the challenging and costly diseases, widely prevalent at a global scale. It is

caused by *Clostridium perfringens* that is normally residing in the intestinal tract of sheep and other animal species (Dikova, 1972; Ganovski *et al.*, 1984; Uzal & Kelly, 1996; Todorov *et al.*, 1998; Aschfalk *et al.*, 2002; Sipos *et al.*, 2003).

There are no literature data about the morbidity rate of enterotoxaemia among mouflons as well as about the role of *C. perfringens* in the pathology of these animals. The observed lethal issues in mouflons with signs characteristic for this disease in our country, necessitated the performance of the present investigations.

### MATERIALS AND METHODS

#### *Animals and specimens*

The investigations were carried out in two intensive breeding game preserves located

in the Stara Planina and Central Rhodopes mountains, respectively. In the first preserve, necropsies of 7 dead mouflons and microbiological examination of three diarrhoeic fecal samples found in vicinity of the feeders were performed. In the other preserve, a *post mortem* examination of the viscera of five healthy mouflons, shot in a hunt was made.

#### *Pathomorphological studies*

Specimens from the viscera were fixed in 10 % neutral formalin. For histopathological examination, the tissues were processed by routine methods. After embedding in paraffin, 4- $\mu$ m cross-sections, stained with haematoxylin/eosin were prepared. Photographs were done with a Logitech Quick Cam and software, fitted to a light microscope.

#### *Microbiological studies*

Bacteriological examination of the three faecal samples and samples from the viscera of 12 mouflons, necropsied within several hours to 24 hours after the death, was done by inoculation in Tarozzi broth (National Centre for Infectious and Parasitic Diseases, Sofia, Bulgaria) and cultivation at 37 °C for 24–48 h. The isolated anaerobic bacteria were identified by microscopic observation of native and Gram- or Foth-stained smears and by detection of the cultural traits.

When *C. perfringens* was detected in the Tarozzi broth, it was further inoculated on Zeissler agar. From single colonies with the specific *C. perfringens* morphology, broth cultures were obtained and tested for production of toxin. For this purpose, the Tarozzi broth was centrifuged at 2000 rpm for 10 min and the supernatant was intravenously injected to albino mice. The typization of *C. perfringens* isolates was done via the toxin neu-

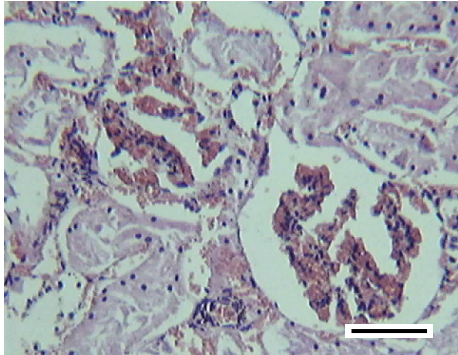
tralization test with type-specific sera for *C. perfringens* types C and D (National Centre for Infectious and Parasitic Diseases, Sofia, Bulgaria) with subsequent intravenous application to albino mice.

For isolation of other microorganisms, inoculations on Mueller-Hinton agar containing 5% blood, Endo agar and Chapek agar (for fungi) (National Centre for Infectious and Parasitic Diseases, Sofia, Bulgaria) were performed. The inocula were incubated at 37 °C for 24–48 h, and agar plates for fungi – for one week at 22 °C. The identification of isolated strains was done by microscopic observation of native and Gram-stained preparations, and by detection of cultural traits on solid and liquid media and biochemical properties using Polymicrotest (National Centre for Infectious and Parasitic Diseases, Sofia, Bulgaria) and the oxidase test. The isolation and identification of bacteria was performed according to the Bergey's Manual (Holt *et al.*, 1994).

## RESULTS

The mouflon population in the Stara planina mountain game preserve station with the observed cases of the disease, consisted of 70 animals. Seven out of them were dead (10%) – 6 male and 1 female at the age of 2–6 years. Four mouflons were found out dead in the winter, and three – in the spring of 2005. The other five shot healthy mouflons were at the age of 6–10 years and showed neither signs of diarrhoea, nor lethality incidence.

The external examination of the dead animals showed a normal weight for the season, extensive staining of the croup and the tarsal joints with soft, dark-green diarrhoeic faeces. A characteristic abdominal finding after the necropsy was the significant amount of transudate, disten-



**Fig. 1.** Acute nephritis in a mouflon with enterotoxaemia. Haematoxylin/eosin, bar = 1000 µm.

ded small intestine with slightly reddened serous coat and thinned mucosa. The large intestine content was soft as well. The liver was cyanotic, the spleen reduced and atrophied, the kidneys – flaccid, soft, cyanotic, with diffusely reddened transverse sections. The lungs of dead mouflons were with pneumonic alterations and oedema. The cardiac muscle was soft and loose.

Histologically, the most characteristic changes were observed in the kidneys of dead mouflons. Hyperaemia, multiple cortical haemorrhages and acute glomeru-

lonephritis were present (Fig. 1). The capsular space was enlarged, the glomerules – hyperaemic with haemorrhages and formation destruction at some loci. The epithelial cells in the renal convoluted tubules were with desquamated or lysed cytoplasm and naked nuclei. In the medullar part, there were also massive haemorrhages and acute tubulonephritis. The spleen exhibited a typical atrophy of the red pulp and the liver was hyperaemic. In the lungs, oedema and haemorrhages were found out. A frequent finding was the focal verminous pneumonia. In some cases, diffuse bronchopneumonia was also found out.

From the bacteriological examination of specimens, *C. perfringens* type C was isolated. As seen from Table 1, it is detected in viscera of both dead and healthy animals. The same type *C. perfringens* was isolated from the diarrhoeic faecal samples found out near to the feeders.

In some specimens positive for *C. perfringens*, other microorganisms were also isolated as followed: from liver samples: single *Corynebacterium sp.* in 1 sample, *Streptococcus pneumoniae* in 1 sample, single *Corynebacterium sp.* and single *Streptococcus pneumoniae* in 1 sample

**Table 1.** Gross pathological and microbiological findings in studied mouflons

Examined mouflons	Number	Isolated <i>Cl. Perfringens</i>					
		Intestinal content		Liver		Other organs	
		Number examined	Number (%) positive	Number examined	Number (%) positive	Number examined	Number (%) positive
With enteritis and pneumonia	7	7	4 (57 %)	7	5 (71 %)	5	5 (100 %)
Healthy shot	5	5	5 (100 %)	5	3 (60 %)	-	-

and single *Staphylococcus sp.* in 1 sample; from lung specimens – *Proteus mirabilis* and single *Pseudomonas cepacia* in 1 sample, *Klebsiella pneumoniae sp. ozaenae* in 1 sample, *Haemophilus sp.* and single *Pseudomonas fluorescens* in 1 sample, *Aspergillus fumigatus* in 1 sample, single *Escherichia coli* and *A. fumigatus* in 1 sample and *S. pneumoniae*, *Staphylococcus haemolyticus* and *A. fumigatus* in 1 sample; in intestinal specimens: *E. coli*, *Enterococcus sp.*, *Bacillus sp.* and *A. fumigatus* in 2 samples, *P. mirabilis*, *P. cepacia* and *P. rettgeri* in 1 sample, *E. coli* and *Enterococcus sp.* in 1 sample and *E. coli*, *Corynebacterium sp.* and *A. fumigatus* in 1 sample.

## DISCUSSION

The results from our pathomorphological and microbiological studies confirmed that the death of mouflons was due to enterotoxaemia caused by *C. perfringens* type C. It is known that the winter/spring seasons and the rapid death of adult animals with gastrointestinal syndrome are typical for this disease in small ruminants.

The enterotoxaemia in mouflons as well as in other domestic and wild animal species is obviously triggered by factors such as change in the feeding pattern, nutritional stress or stress caused by weather conditions. The physiology of feeding in mouflons is very close to that of goats, although with some differences. According to our observations, mouflons are selectively feeding on purer feed, rich in tannins. They prefer the tips of herbaceous plants and bush leaves that decrease the risk of ingestion of grass, contaminated with faeces. Another important fact is that the forest herbaceous plants in the reserves is far less polluted compared to this

on pastures, visited by many different animal species.

Because of its difficult spore-forming capability, the survival of *C. perfringens* in the soil is not prolonged. The infection occurs through a contact with the bacterial pathogen, recently released from another infected animal, primarily by faecal-oral route, especially via direct contact with faeces.

The rearing of mouflons in the closed areas of the game reserve stations is a permanent stress factor and undoubtedly has an impact on the gastrointestinal motility, favourizing the replication of pathogenic clostridia followed by accumulation of their toxins in the intestinal tract. On the other side, the boundaries of the reserves create preconditions for contamination of the environment with bacteria (Haagsma, 1991). The clostridia, causing enterotoxaemia, survive longer in soils with neutral or alkaline reaction. Such is the reaction of the soil during the winter and the spring (Todorov *et al.*, 1998). Probably, this is one of causes for the emergence of these diseases just in this time of the year, because at that time the contamination of feed is more extensive. An important proof, according to verbal communications from the staff, is the detection of diarrhoeic faeces adjacently to feeders 1–2 weeks prior to finding dead mouflons. This fact is further supported by the isolation of the microbial agent from diarrhoeic coprological samples in the affected reserve.

A risk factor is the parasitic background in wild ruminants. According to Tomov (1962) and Uzal *et al.* (1994), the parasitic infections in sheep and goats are important prerequisites for the disease. This is probably valid also in mouflons.

Our results showed that apart the discussed causes, another triggering factor

for the development of enterotoxaemia in mouflons could be *Aspergillus*-caused toxicosis or aspergillosis after consumption of molded hay (in three of studied mouflons), overfeeding with acorns (in one animal), continuous spring rainfalls with abundant green vegetation growth etc. In cases where other microorganisms were isolated from the studied specimens, there were no signs that they could be of etiological importance, apart *A. fumigatus* (mostly in mouflons with bronchopneumonia). This corresponded to the data of Ivanov *et al.* (1967).

The diagnosis enterotoxaemia caused by *C. perfringens* type C as a result of the present studies in mouflons, was confirmed by isolation of the agent from small intestinal content and from viscera, as well as by the detection of the specific toxin. This complies with the statements of some investigators (Tomov, 1962; Sipos *et al.*, 2003) believing that the precise diagnosis of anaerobic infections in small ruminants is not possible without such a bacteriological confirmation. A consistent differentiation from nutritional intoxications is necessary.

In the present studies, *C. perfringens* type D was not detected, but a definite conclusion is possible after studying specimens from more animals. According to a number of authors (Tomov *et al.*, 1965; Ganovski *et al.*, 1984; Todorov *et al.*, 1998; Aschfalk *et al.*, 2002; Sipos *et al.*, 2003) the diseases on domestic and wild small ruminants and pigs, caused by *C. perfringens* types A, C and D are widely prevalent, but most commonly, *C. perfringens* type C is detected. Possibly, mouflons are more sensitive to *C. perfringens* type C, and this perhaps determines its maintenance within the population.

The detection of *C. perfringens* also in viscera of healthy mouflons in the present

studies is indicative for a rapid postmortal penetration and spread of intestinal clostridia.

The present results demonstrate a higher sensitivity of mouflons to *C. perfringens*. This microorganism is evidently part of the intestinal microflora in mouflons as in other species (Sipos *et al.*, 2003; Hattel *et al.*, 2004). The prevention of enterotoxaemia in small ruminants is based on observing a schedule of routine vaccination in adults, as well as on avoiding the predisposing factors for reproduction of the agent in the alimentary tract (Uzal & Kelly, 1996; Todorov *et al.*, 1998). The vaccination in mouflons is hardly applicable and that is why, it is important to prevent the conditions for dysbacteriosis due to impaired nutrition. Another important measure is the regular removal of faeces around the feeders that would significantly reduce the possibility of infection. The supplementation of the diet with probiotics could be used for prevention as well (Seifert & Gessler, 1996).

## REFERENCES

- Aschfalk, B., P. Valentin-Weigand, R. Goethe & W. Müller, 2002. Toxin types of *Clostridium perfringens* isolated from free-ranging, semi-domesticated reindeer in Norway. *The Veterinary Record*, **151**, No 7, 210–213.
- Dikova, T., 1972. Studies on the spread of *Clostridium perfringens* types A, B, C and D in calves and cattle. In: *Proceedings from the Third Symposium of Young Researchers in Agriculture*, Oct. 26–28, Sofia, Agricultural Academy “G. Dimitrov” pp. 133–137 (BG).
- Ganovski, D., T. Dikova & H. Shoilev, 1984. Anaerobic dysentery of pigs. *Veterinary Science (Sofia)*, **21**, No 6, 34–39 (BG).
- Haagsma, J., 1991. Pathogenic anaerobic bacteria and the environment. *Review of Sci-*

- ences and Technics, **10**, No 3, 749–764.
- Hattel, Al., D. P. Shaw, B. C. Love, D. C. Wagner, T. R. Drake & J. W. Brooks, 2004. A retrospective study of mortality in Pennsylvania captive white-tailed deer (*Odocoileus virginianus*): 2000–2003. *Journal of Veterinary Diagnostic Investigations*, **16**, No 6, 515–521.
- Holt, J. G., N. R. Krieg, P. H. A. Sneart, J. T. Staley & S. T. Williams, 1994. In: Bergey's Manual of Determinative Bacteriology, 9<sup>th</sup> edn, Williams & Wilkins, pp. 112–122, 151–252, 534–562.
- Ivanov, I., B. Stamenov, D. Belchev & I. Simov, 1967. The role of *Clostridium perfringens* in the ethiology of enteritis in the new-born calves. *Veterinary Science (Sofia)*, **4**, No 2, 9–14 (BG).
- Nattermann, H., R. Barwisch & L. Briederman, 1990. Die *Bacteroides nodosus*-Infection beim Muflon (*Ovis ammon musimon* Schreber 1782). *Monatsschrift für Veterinär Medizin*, **45**, 882–884.
- Sarrazin, C., J. Oudar, M. Prave, Y. Richard & J. Borel, 1990. First description in the mufelon (*Ovis ammon musimon*) of an enzootic contagious infectious keratoconjunctivitis in the Southern French Alps. *Gibier Faune Sauvage*, **7**, No 4, 389–399.
- Seifert, H. S. & F. Gessler, 1996. Oral long-term administration of probiotic *B. cereus* – an alternative for the prevention of enterotoxaemia? *Deutsche Tierärztliche Wochenschrift*, **103**, No 10, 386–389.
- Sipos, W., L. Fischer, M. Schindler & F. Schmoll, 2003. Genotyping of *Clostridium perfringens* isolated from domestic and exotic ruminants and swine. *Journal of Veterinary Medicine, B., Infectious Diseases*, **50**, No 7, 360–362.
- Takats, A., 1992. Hoof-disease of the mufelon (*Ovis ammon musimon*). *Magyar Állatorvosok Lapja*, **47**, No 9, 475–478.
- Thiry, E., M. Borrens, J. Barrat, J. Blancou & P. P. Pasteuret, 1992. Etude séroépidémiologique des infections à herpesvirus chez les ruminants sauvage en France (*Capra ibex*, *Rupicapra rupicapra*, *Capreolus capreolus*, *Ovis ammon musimon*). *Gibier Faune Sauvage*, **9**, No 1, 87–91.
- Todorov, D., T. Dikova & N. Masalski, 1998. Enterotoxaemia in goats caused by *Clostridium perfringens* type C. *Veterinary Medicine*, **4**, No 2, 95–96 (BG).
- Tomov, T., 1962. Contribution to the spread and season behavior of anaerobic infections in sheep in our country. In: *Proceedings of the Central Veterinary Institute for Infectious and Parasitic Diseases (Sofia)*, No 6, 65–72 (BG).
- Tomov, T., I. Ivanov, K. Sartmadjiev & M. Draganov, 1965. Studies on the spread of *Clostridium perfringens* types A, C and D. *Veterinary Science (Sofia)*, **2**, No 10, 927–932 (BG).
- Tzvetkov, Y. & T. Dikova, 1999. Infection in mufelons, caused by *Corynebacterium pseudotuberculosis*. *Veterinary Medicine*, **5**, No 1, 5–8 (BG).
- Uzal, F. A., M. I. Passini, F. V. Olachea, C. A. Robles & A. Elizondo, 1994. An outbreak of enterotoxaemia caused by *Clostridium perfringens* type D in goats in Patagonia. *The Veterinary Record*, **135**, No 2, 279–280.
- Uzal, F. A. & W. R. Kelly, 1996. Enterotoxaemia in goats. *Veterinary Research Communications*, **20**, No 6, 481–492.

Paper received 26.06.2006; accepted for publication 03.07.2007

**Correspondence:**

Dr. Teodora P. Popova  
Faculty of Veterinary Medicine,  
University of Forestry,  
10 Kliment Ohridski blvd,  
1756 Sofia, Bulgaria,  
e-mail: dr\_tpopova@abv.bg