

Paratuberculosis in wild ruminants in the Czech Republic from 1997 to 2004

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ABSTRACT

Paratuberculosis, a disease caused by the *Mycobacterium avium* subspecies *paratuberculosis*, produces chronic lymphogranulomatous enterocolitis. This worldwide infection affects different domestic and wild animal species. Due to the spread of paratuberculosis on cattle and sheep farms after frequent importation since 1989, the occurrence of paratuberculosis was monitored in seven species of wild ruminants over an eight-year period. A total of 6 935 animals of wild ruminant species were involved: 1 692 red deer (*Cervus elaphus*), 1 534 roe deer (*Capreolus capreolus*), 1 905 fallow deer (*Dama dama*), 125 sika deer (*Cervus nippon*), 1 518 mouflons (*Ovis musimon*), 27 bezoars (*Capra aegagrus*), and 134 chamois (*Rupicapra rupicapra*). These animals were bred in the wild, game parks and on farms. Paratuberculosis was diagnosed in the gastrointestinal tract and/or faeces of 288 animals (4.2% of examined animals). The most infected were red deer (n=206), which are considered more susceptible to this disease than cattle. With respect to the origin of wild ruminants, the highest prevalence of infection was found in deer and mouflon farms (n=250). By RFLP (Restriction Fragment Length Polymorphism) analysis 182 *M. a. paratuberculosis* isolates were classified to eight different RFLP types: B-C1, B-C5, B-C9, B-C16, B-C32, D-C12, M-C16, and R-I4, which we found in domestic ruminants in Europe.

Key words: paratuberculosis, economic losses, molecular epidemiology

Introduction

Paratuberculosis is a serious chronic bacterial disease of domestic and wild

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ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis*, and manifested by diarrhoea and body wasting in infected animals. In wild ruminants, this disease causes losses in animals kept in high densities on farms and in game parks (MACHACKOVA et al., 2004; MACHACKOVA-KOPECNA et al., 2005). Paratuberculosis has been diagnosed in wild ruminants in Europe (POWER et al., 1993; FAWCETT et al., 1995; PAVLIK et al., 2000a; MACHACKOVA et al., 2004) and overseas (DELISLE et al., 1993; MANNING et al., 1998). From the aspect of pathogenesis, the disease is serious due to the long incubation period and potential vertical and horizontal spread of infection at an early age of the animals. The young may get infected at one or two months of age from their mothers by ingestion of infected milk or contaminated feed. However, active shedding of the causative agent and the follow-up of clinical signs pathognomonic of paratuberculosis are not apparent before 8 months of age, but most usually several years later (AYELE et al., 2001).

Paratuberculosis induces a chronic disease in all age groups of deer; the incidence is increased in 8 to 15 months old deer (MACKINTOSH, 1998; MACHACKOVA-KOPECNA et al., 2005). Clinical signs are above all manifested by body wasting and diarrhoea. Gross changes are localized in gastrointestinal tract where granulomatous enteritis and enlargement of adjacent lymph nodes are detected (MACKINTOSH, 1998).

Infected grazing cattle, wild rabbits (*Oryctolagus cuniculus*) and other animals (GREIG et al., 1999; PAVLIK et al., 2000a; MACHACKOVA et al., 2003; MACHACKOVA et al., 2004) may be reservoirs and vectors of this disease under specific conditions in nature.

Paratuberculosis is diagnosed on the basis of culture detection of *M. a. paratuberculosis* in faeces and tissue samples from the gastrointestinal tract. Due to the fact that the causative agent grows slowly, the examination takes two to three months, and in mouflons even longer (PAVLIK et al., 1999; MACHACKOVA et al., 2002). Another possible intravital diagnostics method is serologic examination (methods: complement fixation test – CFT, immunodiffusion test – IDT) for indirect detection of the causative agent of paratuberculosis (based on the production of antibodies). However, from the standpoint of reliability, it is more suitable to use culture examination of faeces for direct detection of the causative agent of infection (PAVLIK et al., 2000b).

In the Czech Republic, paratuberculosis was diagnosed in imported cattle for the first time in 1962 (DRAZAN et al., 1962). Afterwards, only sporadic cases occurred till 1989 (PAVLAS et al., 1997). In the 1990s, the epizootiological situation changed due to the importation of domestic and captive wild ruminants (PAVLIK et al., 2000a), resulting in an increased incidence of paratuberculosis. Accordingly, we carried out a survey of paratuberculosis incidence in 6 935 wild ruminants between 1997 and 2004.

Materials and methods

Gastrointestinal tract samples (mucosa and adjacent lymph nodes of small intestine) or faeces were collected from 1 692 red deer (*Cervus elaphus*), 1 534 roe deer (*Capreolus capreolus*), 1 905 fallow deer (*Dama dama*), 125 sika deer (*Cervus nippon*), 1 518 mouflon (*Ovis musimon*), 27 bezoars (*Capra aegagrus*), and 134 chamois (*Rupicapra rupicapra*). Animals originated from 76 districts of wild nature, 75 game parks and 46 deer and mouflon farms.

Approximately one gram of sample was homogenised by a stomacher and decontaminated in 0.75% HPC (Hexadecyl Pyridinium Chloride: N-cetylpyridinium chloride monohydrate, No. 102340 Merck). The samples (0.2 ml) were cultured in three vials of Herrold's egg yolk medium (HEYM) with Mycobactin J (PAVLIK et al., 2000b).

All mycobacterial isolates were identified by IS900 Polymerase Chain Reaction (PCR). Selected 182 *M. a. paratuberculosis* isolates were analysed by the standardised IS900 Restriction Fragment Length Polymorphism (RFLP) method using the restriction endonucleases PstI and BstEII (PAVLIK et al., 1999).

Results

Paratuberculosis was diagnosed in 288 (4.2%) animals; with red deer (n=206) being the most frequently infected. Infected animals originated from 10 game parks, 10 farms, and 8 regions of wild nature. The highest prevalence of infection was found on deer and mouflon farms (n=250). On the contrary, only 0.6% of free-living animals (n=16) were found to be infected (Table 1).

Table 1. Paratuberculosis in wild ruminants in the Czech Republic during 1997-2004

Species	Type of breeding								
	Farm		Game park		Wild nature		Total		
	No.	Pos.	No.	Pos.	No.	Pos.	No.	Pos.	%
Red deer	914	200	312	3	466	3	1 692	206	12.2
Roe deer	0	0	22	0	1 512	7	1 534	7	0.4
Fallow deer	658	14	1 059	7	188	2	1 905	23	1.2
Mouflon	821	35	554	12	143	4	1 518	51	3.3
Bezoar	0	0	27	0	0	0	27	0	0
Chamois	0	0	0	0	134	0	134	0	0
Sika deer	40	1	85	0	0	0	125	1	0.8
Total	2 433	250	2 059	22	2 443	16	6 935	288	4.2
%	100	10.3	100	1.1	100	0.6			

Pos. = positive culture

Seven different RFLP types (B-C1, B-C5, B-C16, B-C32, M-C16, D-C12, and R-I4) of *M. a. paratuberculosis* were revealed. RFLP type B-C1 predominated in the infected wild ruminant species originating from all husbandry types (Table 2). Even RFLP type B-C1 was found in mixed infection with RFLP types B-C16 and B-C5 in eight farmed red deer (Tables 2 and 3).

Table 2. RFLP types of *M. a. paratuberculosis* in wild ruminants in the Czech Republic

Species	No.	Farm					
		B-C1 B-C5 ^a	M-C16	B-C1	B-C1 B-C16 ^a	B-C16	B-C32
Red deer	133	4	11	78	4	30	1
Fallow deer	16			9			
Mouflon	25			16			
Roe deer	7						
Sika deer	1			1			
Total	182	4	11	104	4	30	1

Explanations:

* not proved contact with infected domestic ruminants, ** contact with infected cattle, a cross-infection with different RFLP types

Table 3. RFLP types of *M. a. paratuberculosis* in wild ruminants in the Czech Republic

Species	No.	Game park			Wild nature			
					No contact*		Contact**	
		M-C16	B-C1	R-I4	M-C16	B-C1	D-C12	B-C16
Red deer	133	1	2				2	
Fallow deer	16		4	1			1	
Mouflon	25		8		1			
Roe deer	7				2	3	2	
Sika deer	1							
Total	182	1	14	1	2	4	1	

Explanations:

* not proved contact with infected domestic ruminants, ** contact with infected cattle, a cross-infection with different RFLP types

Discussion

By comparison of the previously published data concerning prevalence of paratuberculosis in wild ruminants (PAVLIK et al., 2000a; MACHACKOVA et al., 2004), increased numbers of cases of paratuberculosis in red deer, fallow deer and mouflons originating above all from farms were detected. These are usually farms covering an area of less than 10 ha. The main source of infection for captive wild ruminants appears to be fallow deer imported from Hungary in 1989 (PAVLAS et al., 1997) and red deer imported from Scotland in 1995 that were subsequently found positive (PAVLIK et al., 2000a). Moreover, the primary problem of the spread of infection among these herds is uncontrolled transfer of animals without previous serological or culture examination for *M. a. paratuberculosis*.

RFLP analysis of *M. a. paratuberculosis* isolates demonstrated that 6 free-living wild ruminants were infected with identical RFLP type of infectious agent like cattle from the same locality (Table 2). Cases of paratuberculosis outbreaks where *M. a. paratuberculosis* was also isolated from cattle and wildlife, have been described in the Czech Republic (PAVLIK et al., 2000a; MACHACKOVA et al., 2004) and in other countries in Europe (GREIG et al., 1999; BEARD et al., 2001; ALVAREZ et al., 2005). Thus, free-living wild ruminants appear to be infected from domestic or captive wild ruminants. PACETTI et al. (1994) and MARCO et al. (2002) also considered cattle in the pasture to be a potential source of infection of *M. a. paratuberculosis* for wild ruminants.

M. a. paratuberculosis of RFLP type B-C1 is the most common genotype. High *M. a. paratuberculosis* contamination on farms was confirmed by cross-infection of different RFLP types of isolates in our study. MACHACKOVA-KOPECNA et al. (2005) described a similar case of mixed infection with different *M. a. paratuberculosis* RFLP types on one red deer farm. No relationship between the RFLP types and wild ruminant species was found (PAVLIK et al. 2000a).

Our study confirmed interspecies transmission of paratuberculosis and showed that stress and high concentration of wild ruminants kept in unnatural conditions are the main risk factors for spread of the infection and outbreak of the disease.

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SAŽETAK

Paratuberkuloza je kronična limfogranulomatozna upala crijeva uzrokovana bakterijom *Mycobacterium avium* subsp. *paratuberculosis*. Od paratuberkuloze obolijevaju različite vrste domaćih i divljih životinja diljem svijeta. Zbog proširenja paratuberkuloze unosom životinja na farme goveda i ovaca od 1989. godine u Češkoj, uvedena je kontrola zdravstvenog statusa u sedam vrsta divljih preživaca u tijeku 8-godišnjeg razdoblja. U tom je razdoblju pretraženo ukupno 6 935 životinja, i to: 1629 jelena običnih (*Cervus elaphus*), 1 534 srne (*Capreolus capreolus*), 1 905 lopatara (*Dama dama*), 125 sika jelena (*Cervus nippon*), 1 518 muflona (*Ovis musimon*), 27 bezoarskih koza (*Capra aegagrus*) i 134 divokoze (*Rupicapra rupicapra*). Sve životinje su potjecale iz prirodnih uzgoja, parkova divljači ili farmi. Paratuberkuloza je dokazana odgovarajućim pretragama probavnog sustava ili izmeta u 288 životinja (4,2% pretraženih životinja). Najveći broj pozitivnih uzoraka potjecao je od jelena običnog (n=206), koji se i smatra osjetljivijim na zarazu ovom bakterijom od goveda. S obzirom na podrijetlo divljači, najveći postotak zaraze utvrđen je na uzorcima s farmi jelena i muflona (n=250). Metodom

cijepljenja DNA produkata restrikcijskim enzimima (RFLP), 182 izolata *M. a. paratuberculosis* razvrstano je u osam različitih RFLP tipova: B-C1, B-C5, B-C9, B-C16, B-C32, D-C12, M-C16 i R-14. Navedene tipove pronašli smo i u domaćih preživača u Europi.

Ključne riječi: paratuberkuloza, ekonomski gubitak, molekularna epidemiologija
