

# Occurrence of *Mycobacterium avium* subsp. *paratuberculosis* in fecal samples of hunted deer, chamois and ibex in Switzerland

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## Introduction

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiological agent of bovine paratuberculosis, a chronic granulomatous enteritis, also known as Johne's disease. MAP prevalence in domestic food producing animals is increasing globally, causing significant economic losses to the livestock industries through subclinical effects (reduced milk yield and weight loss) and subsequent death of the affected animals. Moreover, several reports suggest a possible link between human Crohn's disease and MAP due to remarkable clinical, epidemiological and pathological similarities (Chacon *et al.*, 2004), but a causal relationship has not yet been proven. Nevertheless, the possible involvement in human disease has led to an increased awareness of these microorganisms as public health concerns.

The natural hosts for MAP are domestic ruminants with higher prevalence in dairy and beef cattle herds around the world (Manning and Collins, 2001). For Switzerland, different studies showed a low prevalence of MAP in the cattle population (Glanemann *et al.*, 2004; Bosshard *et al.*, 2006).

Wild ruminants may act as a reservoir and contribute to the spread of MAP through fecal shedding (Manning, 2011). Their interaction with susceptible farmed ruminants raises the possibility of playing a role in the epidemiology of the disease in domestic livestock. An interspecies transmission between cattle and wild-living red deer (*Cervus elaphus*) has been recently proposed by multitarget genotyping of strains from both animal species (Fritsch *et al.*, 2012). However, reported detection rates in wild ruminants vary among different surveys. From fecal samples of red deer a prevalence of 0.1% has been reported in a Czech Republic survey (Pribylova *et al.*, 2011), whereas a much higher prevalence was found in India where 15% of hog deer (*Axis porcinus*) and 57% of gaur fecal samples (*Bos gaurus*) were tested positive for MAP (Singh *et al.*, 2010).

In view of detection techniques, several culture methods without decontamination or combining chemical or antibiotic decontamination steps and selective media have

been proposed (Glanemann *et al.*, 2004; Akineden *et al.*, 2011). However, these methods are time consuming. Immunological-based systems are faster than culture methods but have a low sensitivity (Glanemann *et al.*, 2004; Collins *et al.*, 2005). Molecular based-methods such as PCR assays may represent a rapid screening system for MAP detection. Most of these protocols are based on the IS900 insertion elements but IS900-like sequences found in other Mycobacteria can lead to false positive results. Therefore other sequences without homologues in other Mycobacteria have been proposed; ISMap02 is present in six copies (Stabel and Bannantine, 2005), ISMav2 is present in three copies (Li *et al.*, 2005), and F57 and Hsp X both occur as single copy genes (Tasara and Stephan, 2005). Nevertheless, these methods have a lower sensitivity compared to the cultural based methods and may not detect subclinically infected animals (low shedders). The objectives of this study were a) to investigate the occurrence of MAP in fecal samples collected from hunted wild ruminants by a combination of cultural detection and PCR based identification and b) to assess the impact of wild ruminants in the spread of MAP in Switzerland.

## Sampling

During September to November 2011, state gamekeepers and hunters collected immediately after shooting and evisceration, *in loco*, fecal samples from wild ruminants. For each animal, sex, age, and location of hunting were recorded. Fecal matter (approximately 10 grams per sample) was collected from the colon after the large intestine had been opened, placed into sterile tubes and sent to the laboratory where samples were stored at –20 °C until processing.

## Detection of MAP

For MAP detection, fecal samples from 3 animals of the same species were pooled (total weight of 6 g). Each

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Table 1: Origin and MAP detection results of the 198 fecal samples collected from wild ruminants during the hunting season 2011.

Species	No. Samples	No. Pools	No. of pools with growth of presumptive positive colonies	No. of F57 PCR confirmed MAP positive samples
Red deer	69	23	11	0
Roe deer	51	17	7	0
Chamois	51	17	1	0
Ibex	9	9	0	0
Total	198	66	19	0

pooled sample was first homogenized in 8 ml of sterile demineralized water and stored under chilled conditions for 4 h. Thereafter, samples were decontaminated using a NaOH/oxalic acid treatment (Beerwerth, 1967). Briefly, samples were mixed in 50 ml of a 4% NaOH solution and subsequently transferred into a sterile centrifugation tube and shaken for 8 min on an orbital shaker (Bühler, Hechingen, Germany). After centrifugation (5010 rpm, 15 min, 20 °C) the pellet was resuspended in 20 ml of a 5% oxalic acid solution and shaken for 15 min on an orbital shaker. Tubes were centrifuged a second time as described before; the pellet was resuspended in 6 ml of sterile 0.9% saline and used as inoculum. For each sample Herrold's Egg Yolk Agar with Mycobactin J and ANV (HEYA) (Becton, Dickinson and Company, Sparks, MD, USA) in duplicates were inoculated with an aliquot (200  $\mu$ l) of the final processed and decontaminated material. Slants were incubated at 37 °C and monitored visually for growth of presumptive positive MAP colonies (small whitish colonies slightly elevated from the surface) every two weeks for up to 16 weeks. Presumptive positive colonies were recorded, subcultured in Middlebrook 7H9 broth supplemented with Middlebrook-ADC growth supplement (Becton, Dickinson and Company, USA) and 2 mg/L Mycobactin J (Synbiotics Europe SAS, Munich, Germany), and incubated at 37 °C for 1 week. After Ziehl-Neelsen staining, subcultures with red acid-fast rods were finally confirmed as MAP by using an F57 sequence-based Real-Time PCR protocol (Bosshard *et al.*, 2006).

## Results and Discussion

In total 198 fecal samples (69 red deer (*Cervus elaphus*), 51 roe deer (*Capreolus capreolus*), 51 chamois (*Rupicapra rupicapra*), and 9 ibex (*Capra ibex*)) were collected. The majority of the hunted animals were on average 2 years old and originated from the central and eastern part of Switzerland. Using the described methods, none of the pooled fecal samples from the wild ruminants were found to be positive for viable MAP (Tab. 1).

Detection was based on phenotypic properties (small whitish colony morphology and slow growth) and Mycobactin J dependency. Culture-positive HEYA slants (MAP-like colonies) were found in 19 out of 66 decon-

taminated fecal pools (11 red deer, 7 roe deer and 1 chamois). None of the ibex samples showed bacterial growth. Red acid fast rods forming cluster from culture-positive HEYA slants were visually detected by Ziehl-Neelsen staining from 10 red deer, 4 roe deer and 1 chamois, respectively, but finally confirmed as non-MAP cells by F57 PCR.

Even though competitive growing microorganisms and chemical treatments might hinder and reduce growth of small numbers of viable MAP cells (Grant *et al.*, 2003), the cultural technique is still considered the "reference standard" for detection of MAP in fecal samples. Based on the results of this first study, a low occurrence of MAP in wild ruminants in Switzerland can currently be postulated. Therefore, wild ruminants do not seem to have a big impact in spreading MAP to farmed ruminants. Nevertheless, further studies covering all regions of Switzerland and including a higher number of animals are necessary to get a complete picture of the impact of wild ruminants in the epidemiology of MAP.

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## References

- Akineden Ö., Fernández-Silva J. A., Weirich S., Abdulmawjood A., Bülte M.: Comparison of two decontamination procedures, three culture media, and real time-PCR assay for the detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) from artificially contaminated raw sausage. Arch. Lebensmittelhyg. 2011, 62: 150–156.
- Beerwerth W.: Die Züchtung von Mykobakterien aus dem Kot der Haustiere und ihre Bedeutung für die Epidemiologie und Bekämpfung der Tuberkulose. Prax. Pneumol. 1967, 21: 189–202.
- Bosshard C., Stephan R., Tasara T.: Application of an F57 sequence-based real-time PCR assay for *Mycobacterium paratuberculosis* detection in bulk tank raw milk and slaughtered healthy dairy cows. J. Food Protect. 2006, 69: 1662–1667.
- Chacon O., Bermudez L. E., Barletta R. G.: Johne's disease, inflammatory bowel disease, and *Mycobacterium paratuberculosis*. Annu. Rev. Microbiol. 2004, 58: 329–363.

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Collins M. T., Wells S. J., Petrini K. R., Collins J. E., Schultz R. D., Whitlock R. H.: Evaluation of five antibody detection tests for diagnosis of bovine paratuberculosis. *Clin. Diagn. Lab. Immunol.* 2005, 12: 685–692.

Fritsch I., Luyven G., Köhler H., Lutz W., Möbius P.: Suspicion of *Mycobacterium avium* subsp. *paratuberculosis* transmission between cattle and wild-living red deer (*Cervus elaphus*) by multitarget genotyping. *Appl. Environ. Microbiol.* 2012, 78: 1132–1139.

Glanemann B., Hoelzle L. E., Bögli-Stuber K., Jemmi T., Wittenbrink M. M.: Detection of *Mycobacterium avium* subspecies *paratuberculosis* in Swiss dairy cattle by culture and serology. *Schweiz. Arch. Tierheilk.* 2004, 146: 409–15.

Grant I. R., Kirk R. B., Hitchings E., Rowe M. T.: Comparative evaluation of the MGIT and BACTEC culture systems for the recovery of *Mycobacterium avium* subsp. *paratuberculosis* from milk. *J. Appl. Microbiol.* 2003, 95: 196–201.

Li L., Bannantine J. P., Zhang Q., Amonsin A., May B. J., Alt D., Banerji N., Kanjilal S., Kapur V.: The complete genome sequence of *Mycobacterium avium* subspecies *paratuberculosis*. *Proc. Nat. Acad. Sci.* 2005, 102: 12344–12349.

Manning E. J., Collins M. T.: *Mycobacterium avium* subsp. *paratuberculosis*: pathogen, pathogenesis and diagnosis. *Rev. Sci. Tech.* 2001, 20: 133–150.

Manning E. J.: Paratuberculosis in captive and free-ranging wildlife. *Vet. Clin. North. Am. Food Anim. Pract.* 2011, 27: 621–630.

Pribylova R., Lamka J., Kopecna M., Trcka I., Moravkova M., Pavlik I.: Mycobacterial screening of Czech red deer (*Cervus elaphus*) populations in overwintering sites, 2004–2006. *J. Wildl. Dis.*, 2011, 47: 780–783.

Singh A. V., Singh S. V., Singh P. K., Sohal J. S.: Genotype diversity in Indian isolates of *Mycobacterium avium* subspecies *paratuberculosis* recovered from domestic and wild ruminants from

different agro-climatic regions. *Comp. Immunol. Microbiol. Infect. Dis.* 2010, 33: 127–131.

Stabel J. R., Bannantine J. P.: Development of a nested PCR method targeting a unique multicopy element, ISMap 02, for detection of *Mycobacterium avium* subsp. *paratuberculosis* in fecal samples. *J. Clin. Microbiol.* 2005, 43: 4744–4750.

Tasara T., Stephan R.: Development of F57 sequence based Real-Time PCR assay for detection of *Mycobacterium avium* subsp. *paratuberculosis* in milk. *Appl. Environ. Microbiol.* 2005, 71: 5957–5968.

Tasara T., Hoelzle L. E., Stephan R.: Development and evaluation of a *Mycobacterium avium* subspecies *paratuberculosis* (MAP) specific multiplex PCR assay. *Int. J. Food Microbiol.* 2005, 104: 279–287.

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