

# Lymphatic vessel puncture of the bovine udder: feasibility for sample collection and detection of *Mycobacterium avium* subsp *paratuberculosis* in the lymphatic fluid by PCR

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## Introduction

Paratuberculosis or Johne's disease (JD), caused by *Mycobacterium avium* subsp *paratuberculosis* (MAP), is a costly and important disease of ruminants in all developed countries. Diagnosis of paratuberculosis is challenging during the early stages of the disease, and currently available laboratory tests do not have satisfactory sensitivity when used to test MAP-infected cows before the onset of clinical signs. The lymphatic system plays a major role in the defence against infection and is an important part of the immune system. The superficial lymph vessels of the bovine udder are accessible for collection of lymphatic fluid by lymph vessel puncture in lactating cows.

The objectives of the study were to: 1) evaluate the feasibility of lymph collection from the bovine udder under field conditions, 2) investigate the potential for detection of MAP in the collected lymphatic fluid of cows infected with MAP, and 3) evaluate lymphatic fluid use as a diagnostic medium for detection of MAP-infected animals in the subclinical stages of JD.

## Materials and Methods

Lymph fluid collection was attempted in 58 cows with varying or unknown MAP-infection status. The reactions of the cows as well as the level of difficulty of

the procedure were recorded in 56 cows. Lymph samples were collected (n=51) and tested for the presence of MAP by nested polymerase chain reaction (PCR). Additionally, these cows were tested for antibodies against MAP in blood samples using a commercial enzyme linked immunosorbent assay (ELISA).

## Results

Lymph vessel puncture caused mild or no signs of discomfort in 94.6% of the cows. Lymphatic fluid was gained on the first attempt in 51.8% of the animals, while sample collection was unsuccessful in 12.1%. Overall, MAP was detected in 43.1% of the lymph samples, with 66.7% positive results for cows with clinical JD, 42.8% positive results for asymptomatic cows with a concurrent positive or suspicious ELISA result, and 38.7% positive results in cows with a negative ELISA result.

## Significance

The results of the study demonstrate that the puncture of lymphatic vessels of the udder of lactating cows is well tolerated by the most cows and can be easily performed on the farm. The isolation of MAP from lymph fluid by PCR indicates that this approach holds promise for the early detection of JD in lactating dairy cows.