

## Incidence of *Mycobacterium avium* subspecies *paratuberculosis* in bulk-tank milk samples from different regions in Switzerland

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*Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a pathogen causing chronic inflammation of the intestine in many animals, including primates, and is considered to be implicated in Crohn's disease in human beings (Hermon-Taylor and others 2000). Crohn's disease is a chronic granulomatous ileocolitis of humans, with significant similarity to paratuberculosis. High herd prevalences of MAP have been found in dairy cattle in particular (Thorne and Hardin 1997). A recent USDA survey indicated that 41 per cent of dairy herds in the USA have at least one MAP ELISA-positive animal. Roughly 22 per cent of all dairy herds in the USA have at least 10 per cent of animals testing positive (Collins 1998).

It has long been known that MAP can be cultured from the milk of cows clinically infected with paratuberculosis (Taylor and others 1981). Recent work has shown that MAP can also be cultured from the milk of apparently healthy, subclinically infected cows (Sweeney and others 1992, Streeter and others 1995). Moreover, thermal tolerance data indicate that this organism is more heat resistant than other *Mycobacterium* species and is capable of surviving the pasteurisation of raw milk (Chiodini and Hermon-Taylor 1993, Grant and others 1998, Sung and Collins 1998).

A resistance to pasteurisation and a possible causal connection to Crohn's disease means that MAP is a significant food-safety issue. The aim of this study was to collect prevalence data of MAP in bulk-tank milk samples from different regions in Switzerland and to compare these results with existing data from the literature.

A total of 501 raw milk samples originating from three different regions in Switzerland (north-east, central and north-west) were screened for the presence of MAP. The samples were collected between February and April 2000, and February and March 2001, and were kept frozen until investigation. Samples of milk (10 ml) were mixed with 100  $\mu$ l Triton X-100 (Calbiochem) and centrifuged at 1600 g for 30 minutes. The resulting pellet was then transferred into blue ribolyser tubes (Hybaid), resuspended in 400  $\mu$ l mycobacterial lysis buffer (2mM ethylenediamine tetraacetic acid [EDTA], 400mM sodium chloride, 10mM Tris-HCl [pH8.0], 0.6 per cent sodium dodecylsulphate, 331 ti/ml proteinase K) and incubated overnight at 37°C. The samples were centrifuged at 6.6 m/second for 45 seconds in a ribolyser (Hybaid) to facilitate DNA extraction. DNA was then extracted with phenol/chloroform/isoamylalcohol (25:24:1) and precipitated with 3M potassium acetate for 30 minutes at -70°C, centrifuged (13,000 g for 15 minutes), washed in 70 per cent ethanol, dried and resuspended in TriS-EDTA buffer.

A sample of the resuspended DNA (5  $\mu$ l) was used for an isgoc nested PCR specific to MAP. The primers p90 5'-GAAGGG TGTTCCGGGGCCGCTGCTAGG-3' and p91 5'-GGCGTTGAGGTCGAT CGCCCACGTGAC-3' were used for the first 30 amplification cycles in accordance with Hermon-Taylor and others (2000).

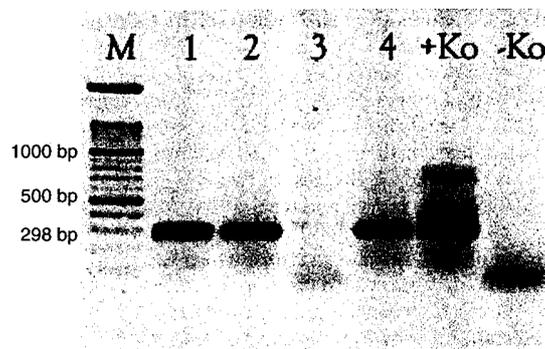


FIG 1: Agarose gel electrophoresis of KR products obtained from different bulk-tank milk samples following the nested IS900 PCR. Lane M 100 by molecular weight ladder (Roche Diagnostics), Lanes 1, and 4 Positive samples with a specific amplification product 298 bp, Lane 3 Negative sample, +Ko Positive control with IS900 nested product of 298 by in length and additional higher molecular weight amplification product 350 bp, 400 by and 704 bp, indicative of strongly positive samples, -Ko Negative control

The PCR mix consisted of 50  $\mu$ l reaction volume containing a final concentration of 2  $\mu$ M of each primer, 2.5mM magnesium chloride, 100  $\mu$ M deoxyribonucleoside-5'-triphosphate (dNTP), 2 U Taq polymerase (Promega) in 1 x reaction buffer (Promega). Reactions were cycled as follows: 94°C for five minutes (one cycle); 94°C for one minute, 58°C for one minute, 72°C for three minutes (30 cycles); and 72°C for seven minutes (one cycle).

A 2  $\mu$ l sample from the primary amplification was then used with the primers AV1 5'-ATGTGGTGTGCTGTGGATGG-3' and AV2 5'-CCGCCCAATCAACTCCAG-3' for the nested PCR. The PCR mix consisted of 50  $\mu$ l reaction volume containing a final concentration of 2  $\mu$ M of each primer, 1.5mM magnesium chloride, 100  $\mu$ M dNTP, 2 U Taq polymerase in 1 x reaction buffer. Reactions were cycled as follows: 94°C for five minutes (one cycle); 94°C for one minute, 58°C for one minute, 72°C for three minutes (40 cycles); and 72°C for seven minutes (one cycle). PCR products were visualised on 1.5 per cent agarose gels (Eurobio). The IS900 nested product is reported to be 298 by in length but a strong signal would also give non-specific bands at 350 bp, 400 by and 700 by (Hermon-Taylor and others 2000).

Of the 501 examined samples, 112 (22.4 per cent) were PCR positive (Fig 1). The prevalence of MAP in the different parts of Switzerland was between 7.5 per cent and 36.7 per cent. Sixty-nine (22.3 per cent) of 309 bulk-tank milk samples from north-east Switzerland, seven (7.5 per cent) of 94 samples from central Switzerland and 36 (36.7 per cent) of 98 samples from north-west Switzerland were IS900 PCR positive.

There are no comparable data on the occurrence of MAP in milk samples in Switzerland. Two earlier studies investigated serology of Swiss milk cows using ELISA. Meylan and others (1995) described a seroprevalence of 4.9 per cent by examining 595 animals in one region in Switzerland. This study, however, was concerned with animal-level prevalence which, compared with herd-level prevalence, is generally lower. Stark and others (1997) found a herd-level prevalence of 8.0 per cent by investigating 113 farms. However, no information was given about the geographic distribution of positive herds and the result is, therefore, not directly comparable with the present study.

Some data on the prevalence of MAP in raw milk samples are available from other countries. Many studies, however, examined milk from clinically ill cows. Furthermore, there are considerable difficulties in obtaining comparable data on prevalence of MAP, because a variety of diagnostic methods (culture, ELISA, PCR) have been used. Prevalence of MAP in milk from clinically ill cows varies between 5 and 55 per cent (Taylor and others 1981, Giese and Ahrens 2000) and in milk samples of subclinically infected cows between 2 and 12 per cent (Sweeney and others 1992, Streeter and others 1995, Jakobsen and others 2000). Nielsen and others (2000) examined bulk-tank milk samples from six different regions in Denmark. They performed an ELISA test on 900 bulk-tank

Veterinary Record (2002) 150, 214-215

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milk samples and detected an apparent herd-level prevalence of 47 per cent (423 MAP antibody-positive samples). In a study by Millar and others (1996), 7 per cent, overall, of pasteurised whole cows' milk samples obtained from retail outlets throughout central and southern England tested positive for MAP by IS900 PCR as well as by culture. It was also noted that there was a conspicuous seasonality from January to March and between September and October in the frequency of samples testing positive, when up to 25 per cent of samples were affected.

Further research will be necessary using milk samples collected from all over Switzerland to investigate possible geographic clustering. Moreover, MAP strains must be isolated in order to characterise isolates genotypically. Pasteurised milk samples collected from retail sources should be examined for MAP by culture method, in order to evaluate the effectiveness of pasteurisation technologies.

## ACKNOWLEDGEMENTS

The authors would like to thank H. Dahler, H. Risi and H. Ringgerberg, the Milk Inspection Service, Switzerland, for help with collecting the samples, as well as J. Hermon-Taylor and T. Bull, St George's Hospital Medical School, London, for providing details of the IS900 nested PCR method.

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## ABSTRACTS

### Effectiveness of meloxicam for treating cats with painful locomotor disorders

SIXTY-NINE cats were allocated to two groups balanced for age, weight, sex, and the duration and severity of the clinical signs of pain due to acute or chronic locomotor disorders. One group was treated with meloxicam drops (0.3 mg/kg orally on the first day and 0.1 mg/kg for the next four days), and the other group was treated with ketoprofen tablets (1 mg/kg orally once a day for five days). Both treatments produced significant improvements in the cats' demeanour, feed intake and weight-bearing, and significantly reductions in their degree of lameness, signs of pain on palpation and inflammation. There was no significant difference between the two treatments in these respects, but meloxicam was significantly more palatable than ketoprofen.

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### Vibration increases femoral bone density in sheep

ADULT ewes which stood on a platform which vibrated at a high frequency for 20 minutes each day for five days a week over a one-year period increased their femoral bone density by 32 per cent. Bone trabeculae were also shown to have closer spacing, which is consistent with stronger bone. Histomorphometric studies of bone turnover suggest that this effect may be due to the increased (more than two-fold, but not statistically significant) bone formation and mineralisation. However, there were no changes in cortical bone. The load applied to the bone from this level of vibration was about 5 microstrain, which was much less than the load sustained during roaming of the pasture, which the animals did (treated and controls) the rest of the time.

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