PREVALENCE OF JOHNE'S-DISEASE INFECTION IN CATTLE IN UMBRIA, ITALY.

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SUMMARY

A total of 788 serum samples from dairy cattle in Umbria, Italy, were tested for the presence of antibodies to Mycobacterium avium subspecies paratuberculosis (MAP) using a commercial enzyme-linked immunosorbent assay (ELISA) kit. The sampled animals came from 19 herds representative of the central area of the Umbria County (Perugia and Assisi districts). Using the manufacturers suggested cut-off for a positive test, 44 animals (5.6%) were positive. Using the sensitivity and specificity claimed by the manufacturer of the ELISA kit, the true prevalence in Umbria dairy cattle overall was calculated as 9.7% (99% CI, 7.0%, 12.4%).

Key words: paratuberculosis, Johne's disease, Crohn's disease

INTRODUCTION

Johne's disease is a chronic debilitating disease of ruminants caused by the bacterium *Mycobacterium avium* subsp. *paratuberculosis* (MAP). This disease has gained increased notoriety in recent years because of concerns about a possible relationship to gastrointestinal diseases of humans. The increased awareness of Johne's disease in the dairy community also has been fostered by reports of the negative impact of Johne's disease on milk production and the overall economic health of dairies. There have been discussions in Europe of disease control programs on a state-by-state or even national level and on the possible links between Crohn's disease and paratuberculosis *(2)*. A possible association between *Mycobacterium avium* subsp. paratuberculosis (*M. paratuberculosis*) and Crohn's disease was first suggested in 1913, when similarities between the gross pathology and symptoms of Johne's disease in cattle and those of Crohn's disease in humans were first noted. Crohn's disease is now generally believed to have a multifactorial aetiology with genetic predisposition, environmental factors (infectious agent, diet or smoking), and abnormal inflammatory response all playing a part. Evidence supporting a link between M. paratuberculosis and Crohn's disease includes: higher detection rates of M. paratuberculosis by PCR and culture in gut samples from Crohn's patients compared to controls; demonstration of a serological response to M. paratuberculosis antigens in Crohn's patients; and anti-M. paratuberculosis antibiotic therapy resulting in remission, or substantial improvement in disease condition, in many patients *(4)*. The available scientific evidence has been reviewed by a number of expert groups in recent years. The consensus opinion, at present, is that the available information is insufficient to prove or disprove that M. paratuberculosis is the cause of Crohn's disease, but the hypothesis is still plausible. The recent discovery of a susceptibility gene in Crohn's patients, NOD2/CARD15, does not preclude a role for M. paratuberculosis in the pathogenesis of at least some cases of Crohn's disease, since the function of this gene is bacterial sensing in the gut. If *M. paratuberculosis* does contribute to the causation of Crohn's disease then it may not be acting as a conventional infectious agent. To establish program effectiveness, baseline prevalence data are needed (especially for different geographic areas and management schemes). Surveys for MAP are difficult to accomplish because of the chronic nature of the infection and the lack of good testing methods for animals in the pre-clinical stages. Surveys done in USA in the past typically have examined limited geographical regions and reported sero-prevalence up to 7.29% of cows and of 50% of herds and faecal-culture prevalence of 3.05%. The prevalence compare to herd-level prevalence of 18 and 16.7% in Belgium and the maritime provinces of Canada, respectively. In Austria, individual-animal prevalence in Holstein cattle was reported 3 or 8% depending on the enzyme-linked immunosorbent assay (ELISA) technique used and Belgium had a reported individual-animal prevalence of 0.87% *(1)*. Management styles and facilities differ widely in the dairy industry. Smaller herds often use pasture grazing for an important percentage of the diet. The Assisi district features some of the earliest dry-lot dair-

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ies in the county. These dairies use total-mixed rations comprised of a variety of commodities as well as forage crops fed in bunks. These dairies often raise the calves in areas of the dairy that are entirely separate from the milking herd - or (in about 50% of the herds) delegate raising and milk feeding of the calves to offsite calf raisers. The central part of the county (Perugia and Assisi districts) has the largest population of dairy cattle, with approximately 5,000 dairy cows. The whole region has a population of 10,000 dairy cows. The dairies in this area are typically newer free-stall dairies that have many management characteristics in common with the dairies in the northern part of Italy. The herds frequently purchase replacement cows and heifers as well as bulls. Therefore, these dairies have a high risk for bringing animals infected with Johne's disease into the herds. Dairies in these regions also tend to cull (less than 15% annually). With many cows >5 years old in these herds, the herds tend to have relatively high clinical Johne's disease. We report a study to determine Johne's disease-MAP sero-prevalence in dairy cattle in Umbria, Italy.

MATERIALS AND METHODS

Nineteen dairies in the Perugia and Assisi districts were visited and 788 blood samples collected during January-September 2006. The 19 dairies were randomly selected using premise-identification numbers dairy database at the onset of the study (specifically all numbers for given region were printed, cut out and drawn from a "hat"). These dairies were selected to represent the central Umbria dairy industry spatially, therefore more dairies were selected from areas of higher dairy density. With approximately 350 dairies and 10,000 cows in Umbria, this sample size provides a 99% confidence level for an expected countywide cow-level prevalence down to 0,56% (±1.5%) and a 99% confidence level for a countywide herd-level prevalence down to 20% (±10%) (Win PEPI 5.5 http://www.brixton-health.com). Depending on the area region involved, the subset of dairies used in this study had Johne's-disease-related management practices that were variably similar to those of the larger group of dairies sampled. All dairies were milking >50 cows. The samples were tested according to manufacturer's specifications with a commercial Johne's-disease ELISA kit (HerdChek Mpt, IDEXX Laboratories Italia SrL, Milano, Italy). Samples initially were tested in single wells and any samples with an S/P ratio between 0.15 and 0.30 were re-tested using the double-well format. In re-tested samples, the positive, negative or suspect classification was based on the results of the double-well test. In the kit insert, the manufacturer states that the "test shows a sensitivity in excess of 50% and specificity above 99%". True prevalence was calculated using standard methods (http://www2.unipr.it/~bottarel/epi/HomePage.html).

RESULTS

Of the total of 788 cows sampled, 44 (5.6%) were positive using an S/P ratio of 0.30 as the cut-off point (as suggested by the manufacturer of the ELISA kit). Using a sensitivity value of 50% and a specificity of 99% (as claimed by the manufacturer) the true prevalence in Umbria dairy cattle overall was calculated as 9.7% (99% CI, 7.0%, 12.4%). Experience suggests the sensitivity and specificity claimed by the manufacturer are optimistic. If sensitivity and specificity for the ELISA kit of 27% and 90% are assumed, then the true prevalence in Umbria dairy cattle overall is instead 18% (99% CI, 16.3%. 19.7%) and the true prevalence values are calculated as 14.6 (99% CI, 18.1%, 21.7%). Using the manufacturers suggested cut-off point, and if an individual herd is classified as positive based on having one or more sero-positive animals, then 9 (47.4%) of the herds in the area would be classified as positive. Using a test with a specificity of <100% and repeated testing results in increased chance of falsely identifying an animal and, by extension, a herd as positive. Therefore, it might be more appropriate to classify a herd as positive only if multiple animals are seropositive. When this more-rigorous standard was used (≥2 seropositive animals) 6 (31.6%) of the herds were classified as positive.

DISCUSSION

The sero-prevalence of Johne's disease we found is within range of apparent prevalence previously reported for Italy and other parts of the EU *(2, 5, 7)*. Aggressive culling practices (like those used in northern Italy dairies), might mitigate some economic effects *(6)*. Until in-depth studies of the epidemiology and economics of Johne's disease in dairies are performed, it remains to be determined whether MAP has a large-enough economic impact to justify a mandatory state-wide control program (rather than the current voluntary strategy that relies on individual producers to decide if Johne's disease is a significant problem that justifies aggressive control strategies in their own herds) *(8)*. It has long been known that *Map* can be cultured from raw milk of clinically infected cows with paratuberculosis. It is probable that the organisms are present within the monocyte population abundant in milk, but they also occur extracellularly. More recent work has shown that *Map* can also be cultured from the milk of apparently healthy subclinically infected cows *(2)*. Clinically infected animals may shed up to 1012 *Map* per ml in

their faeces. Subclinically infected animals also shed the organism though usually in lower amounts. Infected dairy cows and sheep shed *Map* in their milk *(9)*. Given the high prevalence of *Map* in the dairy herds and domestic livestock of western Europe and North America, it is inevitable that *Map* will from time to time be present in bulked tank milk being brought to pasteurisation plants. This raw milk is normally subjected to treatment either by pasteurisation, UHT treatment or sterilisation. The proportion of milk consumed in each of these categories varies considerably throughout Europe. Because of the difficulty in culturing the organism, it has proved problematic to experimentally determine the efficiency of treatments such as pasteurisation. Several studies have shown that *Map* prepared in *in vitro* cultures, spiked into whole cows' milk at a range of microbial concentrations and then treated with experimental pasteurisation, could still be cultured from some samples, after exposure to 65oC for 30 minutes (the Standard Holder method) or 72°C for 15 seconds (the High Temperature Short Time method) *(2, 10)*. These studies have been criticised principally on the grounds that experimental pasteurisation does not accurately reproduce the conditions such as turbulent flow which occur in commercial pasteurisation units. In addition, because of the presence of the organism in monocytes, spiking studies may not be representative. A recent interim report of ongoing work in the UK has reported the finding of viable *Map* in around 5% of raw milk samples examined and in about 3% of pasteurised samples examined, although no information is yet available on the numbers of organisms present *(3)*. Because of its presence in raw milk, *Map* may be initially present in cheeses made from raw milk from infected animals, or in those made from milk exposed to pasteurisation at lower temperature prior to the cheese-making process. MAC (*Mycobacterium avium* complex) are generally resistant to acid conditions and are known to be able to resist the acidic conditions created in intracellular phagolysosomes as part of their strategy for survival within the host cell. MAC may thus survive low pH conditions involved in cheese-making, although the recovery of MAC from cheese, by growth in conventional culture, may be unreliable. At the present time there are no publications from laboratory based research specifically addressing the question of whether *Map* is present in cheese, but epidemiological studies for environmental risk factors for MAC in patients with HIV disease, identified an increased risk associated with the consumption of hard cheeses *(3)*. Macrophages containing *Map* are known to be found throughout the body of animals with the advanced pluribacillary form of Johne's disease. Most tissues including lymph nodes, spleen, bone marrow, liver, kidney and lung are affected but titres of bacteria are low.

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ZUSAMMENFASSUNG

PRÄVALENZ DER JOHNE-KRANKHEIT BEI RINDVIEH

Insgesamt 788 Serummuster, genommen von Milchrindvieh in Umbria/Italien, wurden auf Anwesenheit von Antikörpern auf Mycobacterium avium subsp. paratuberculosis (MAP) mit Hilfe von kommerziellem ELISA Test (Immunoenzymtest) geprüft. Die gemusterten Tiere wurden auf 19 Farmen in mittlerem Teil der Provinz Umbria (Kreise Perugia und Assisi) gezüchtet. Nach Anweisung des Herstellers benutzte man den Grenzwert für positiven Test, so wurden 44 positive Tiere (5,6 %) festgestellt. Auf Grund der Empfindlichkeit und der Spezifität des ELISA Testes, die der Hersteller anführt, wurde ausgerechnet, dass die gesamte Häufigkeit der Krankheit bei Milchrindvieh in Umbria wirklich 9,7 % betrug (Grenzen der Zuversichtlichkeit 99 %, 7,0 %, 12,4 %).

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QUALITATIVE AND QUANTITATIVE CHARACTERISTICS OF NEW ZEALAND WHITE RABBIT MEAT

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SUMMARY

New Zealand white rabbits were used as a material for the study. Rabbits were fed ad libitum with commercial pelleted feed, with the addition of small amounts of green feed and hay. The experiment included 30 rabbits, 15 males and 15 females. Previously defined live weight of 1800 to 2000 g was achieved within 75 days and after that they were immolated. Study results presented in this paper show fattening and slaughter properties of New Zealand white rabbit, as well as meat composition and meat. The achieved average weight of male rabbits was 1963.67 g and meat-to-bone ratio was 44.93%, 1907.00 g and 45-08% respectively in female rabbits. Average participation of the lower part of the body in male/female rabbits was 33.27% / 32.34%, back part 34.90% / 34.43%, and front part 22.57% / 22.45%. Average water content in the meat of male/female rabbits was 74.93% / 74.39%; protein content 22.02% / 21.79%; fat content 0.48% / 0.96%, and content of the minerals 1.26% / 1.17%.

Key words: *New Zealand white rabbit, meat quality*

INTRODUCTION

Constant growth of human population all over the world imposes the obligation of intensification of food production, primarily of biologically most valuable proteins. Rabbit breeding in this concept of food production is very important. A large number of pure and hybrid breeds are used in the production of rabbit meat. New Zealand white rabbit is the prevailing breed in Bosnia and Herzegovina, and it is used exclusively for meat production.

In general, basic characteristics of rabbits are high fertil-

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