The Paratuberculosis Newsletter

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Notes from the Editor

The level of activity on paratuberculosis globally is believed to be quite high. Yet, there has been limited with contributions to this issue of the Newsletter. I bet you are just fine-tuning your contribution, but there can also be too much fine-tuning.

I have received only one contribution, and again from my most faithful contributor Gilles Monif. Thank you Gilles. Where are the comments, short reports etc. from the rest of you? Because of the limited information provided, I took the liberty to include a summary of some of my own work in this issue, and I due apologise for that. However, to avoid such unpleasantness in the future, please share your work or your ideas with the rest of the society.

The new year is just around the corner, and 2010 will hopefully be the year, where there will be so much to read in the Newsletter that your boss will start shouting at you for spending all the time reading interesting findings and stories. I will wish you all a pleasant December and a Happy New Year.

DEADLINE FOR NEXT ISSUE: February 15, 2010.

All contributions should be sent to ssn@life.ku.dk

Søren Saxmose Nielsen
Editor

1. Short communications

Paratuberculosis in Dairy Cattle – Epidemiological studies used for design of a control programme in Denmark

Søren Saxmose Nielsen

Summary of dr.med.vet. thesis

Mycobacterium avium subsp. *paratuberculosis* (MAP) causes chronic infections in dairy cattle worldwide, resulting in economic losses to dairy farmers. This infection has existed for many years. Its control is hampered by inaccurate diagnostic tests. These tests are not very sensitive tools for detecting MAP-infected animals due to the chronic nature of the infection. MAP infections can be insidious, and economic losses may only be realised after the bacteria have been present in a herd for many years.

The aim of this thesis was to describe a cost-effective, risk-based approach to control of MAP infections in dairy herds. Specifically, epidemiological studies were conducted to a) characterise the performance of diagnostic tests; b) establish prevalences; and c) identify risk factors. The information was then compiled in the description and evaluation of a control scheme. This work is described in seven chapters, which are supported by studies described in thirteen accompanying papers.

The background of the thesis was: Diagnosis of MAP infections can be obscured by low specificity and low sensitivity, resulting, respectively, in false-positive and false-negative test results. MAP belongs to *M. avium*, which has four subspecies and is widespread in the environment. Non-MAP *M. avium* infections may result in false-positives in tests used to detect MAP infections, reducing test specificity.

The sensitivity of most tests is affected by the chronic nature of the infection. Infections can be latent for an unknown period of time. Relatively little information is available on infection dynamics and the diagnostic test responses in the course of a MAP infection, because only a few longitudinal studies have been carried out. The exact timing of infection is unknown, although MAP infections are primarily thought to occur in calfhood. In addition, it is unclear whether all infections result in adverse effects, such as bacterial shedding, reduced milk yield, weight loss and eventual death. Nor is it clear whether pro-inflammatory immune reactions clear the infection or manage to keep it under control. Because end-stage disease can occur at any time during an animal's life, the incubation period appears to be variable.

A review of the accuracies of diagnostic tests revealed large variation in the performance of various ELISAs and faecal culture. However, results of the present thesis showed that distinction between different stages of infection involving the categories "MAP-infected", "MAP-infectious" and "MAP-affected" increased the ease of interpretation. The diagnostic tests are not particularly sensitive in detecting infected animals. However, immunity-based diagnosis relative to adverse effects may be a useful way to control MAP infections, because the occurrence of humoural immune responses seems to correspond with deterioration of the infection. If the tests are used in relation to adverse effects, they should be evaluated in relation to each purpose. The studies in the review were all cross-sectional, but an indirect antibody ELISA for detection of MAP-infected and MAP-infectious animals in a longitudinal study design was characterised in this thesis.

The results suggested that sensitivity in the detection of MAP-infected animals increased with age, whereas the probability of detecting MAP infectious animals was less affected by the age of the tested animal. It was also demonstrated that most cows shedding MAP would test positive in the antibody ELISA at some point in time. Furthermore, it was demonstrated that antibodies to MAP occurred 3–4 years prior to the detection of MAP shedding by faecal culture in approximately 20% of cows classified as high shedders. Antibodies may therefore occur relatively early in some infected animals. The results also

suggested that testing animals just once, one year before detected shedding would result in the detection of only 30% of the animals that became high shedders. Testing in the interval 0–3 months prior to onset of detectable shedding would result in detection of up to 60–70% of the high shedders. The longitudinal studies also suggested that in general ELISA may result in positive tests prior to onset of detectable bacterial shedding. Higher ELISA values are equivalent to a higher probability of MAP shedding. Finally, it was demonstrated that the milk yield of animals with fluctuating antibody reactions can be higher than that of repeated test-negative herd-mates. Animals with the last milk sample being positive or repeated positive ELISA-tests can display a significant drop in milk production. Therefore, frequent testing and ordinal ELISA values can be used to detect animals with a high risk of MAP shedding and, in combination with milk production data, can be used to determine which animals should be considered for culling.

ELISA is not a very sensitive detector of MAP-infected animals. Yet it has been the test most widely used to estimate prevalences. A review of prevalences in Europe suggested that most prevalence estimates are not comparable, partly as a result of the variable test sensitivity estimates. Therefore, it is difficult to estimate within-herd prevalences and certify herds free of infection. A Bayesian mixture model was developed. It was found to be more precise than a cut-off based model. The mixture model was subsequently used to assess management factors associated with the development of antibodies to MAP.

To control MAP infections, information about transmission is needed. The transmission of MAP is thought primarily to occur via the faecal-oral route, but MAP can also be transmitted via infected milk or in utero. A retrospective and a prospective study were conducted to assess risk factors for transmission of MAP. It was demonstrated that the ELISA status of a dam influenced the ELISA status of her calf. Calves fed colostrum from multiple cows rather than just one cow had a higher risk of having MAP antibodies as an adult. In addition, the “source of milk” only appeared as an important risk factor when calves were suckling milk from a foster cow. Housing type and animal density of calves and young stock appeared to affect the risk of developing antibodies. The identified risk factors along with conceptual and previously identified risk factors suggest that efforts to control MAP should include measures to ensure that the manure from adults is kept from susceptible animals (mainly calves). Furthermore, milk should be considered infectious, and in utero infections are likely to occur.

Specific management practices can be adopted to deal with the risks mentioned above (e.g. calves can be removed from their dam immediately after birth). However, in many farming production systems these practices are considered time-consuming and therefore will not be implemented. A risk-based system that is tailored to each farm may be an acceptable option to herd managers.

A risk-based system with identification of high- and low-risk animals was investigated as a cost-effective solution requiring fewer animals to be managed. Testing with milk ELISA would be conducted four times per year to identify high-risk animals. Special management procedures to avoid transmission from high-risk animals should be established; low-risk animals can be given less attention. High-risk animals thought to contribute to high bacterial loads in the environment should be culled prior to next calving. Simulation studies indicated that in production systems, where a high workload is required to reduce the risk of transmission of MAP at calving, a risk-based approach would be cost-effective. A non-risk-based approach would be more cost-effective in systems where all animals are managed with high bio-security standards at low cost. A drawback of the latter would be that no data are available to monitor changes in MAP prevalence.

There are still insufficient data to show proof of concept, but frequent testing has previously proven to be a useful strategy for a chronic insidious infection – the infection being tuberculosis caused by *M. bovis* in cattle. Certification of freedom from MAP in a herd is not yet possible owing to the low sensitivities of tests detecting MAP infections, but continued monitoring with ELISA may provide historical data which, in a Bayesian framework, can be used to increase the estimated probability that a herd is free of MAP.

The results in this thesis demonstrate that, used appropriately, milk ELISA can be a cost-effective method for controlling MAP infections in dairy cattle. Frequent testing is necessary, along with changes in management designed to reduce transmission. The role, in MAP transmission, of further management factors should be explored in order to optimise control schemes.

Note: A limited copies of this thesis will be available (free of charge) if you send me your postal address to: ssn@life.ku.dk However, it will be on a first-serve basis, so please let me know as soon as possible if you are interested

2. Comments & Opinions

An ounce of prevention is worth more than a pound of cure: Certificates of animal health must be just that

Gilles R. G. Monif

Containment of disease is central to USDA's mission; yet, the transport of animals across state and national boundaries requires only a certification of apparent health of the animal.

While effective steps had been taken in the United States to curtail *Mycobacterium bovis* (bovine tuberculosis) in dairy and beef cattle, cattle in Latin American countries still have bovine tuberculosis and paratuberculosis co-existing within a given herd. According to the Animal and Plant Health Inspection Services Audit Report (Report No. 50601-0009-Ch September 2006, between fiscal years 2001 and 2005, 75% of bovine TB cases detected through slaughter surveillance were determined by APHIS to have originated from Mexico. Mexico annually exports one million cattle to the United States.

Mexico reported over 2,000 *M. bovis* infected herds. Over 95% of the cattle imported from Mexico spend from 5 to 14 month on U.S. premises prior to slaughter. The chronic draught conditions in Texas and the resultant cost of feeding dairy and beef cattle has forced the relocation of animals to other dairy and beef states. Draught conditions place animals under added environmental and, in some cases, nutritional stress. In such circumstances, animals with prior marginal immunological governance of their mycobacterium infection tend to reactivate their infection.

The state of Nebraska is currently fighting to preserve its bovine tuberculosis-free status. Herds in the north-central and Sandhill regions of Nebraska have been quarantined pending testing. None of the quarantined cattle can be sold unless owners receive a special permit for immediate slaughter. Value of calves in now-quarantined herds will be, more probably than not, downgraded even if their test status is negative. Negative dairy and beef herds having fence-line contact with infected herds and negative herds in which a single animal was recently purchased from an infected herd become subjected to quarantine. If a herd is found to harbor *M. bovis* infected cows, the policy has been to destroy the entire herd.

The prevalence of bovine tuberculosis is destined to increase. As infected animals are identified in new herds, animals from these herds will have already been shipped to other states or countries. Some Nebraska cattle, potentially exposed to tuberculous animals before the herd was quarantined, had already been sent to Colorado and South Dakota.

Veterinarians can no longer do "over-the-fence" inspection of cattle. If he or she certifies an animal as being healthy and that animal is subsequently documented to have significant Map infection, he or she has bought the animal. If he or she certifies an animal as being healthy and that animal is documented to have bovine tuberculosis, he or she has bought the entire herd.

USDA and state agencies need to address some of theoretical issues pertaining to animal certificates of health. A certificate of health should clearly delineate the animal's current *M. bovis* and *M. avium* subsp. *paratuberculosis* status, particularly in cattle originating in Mexico and being shipped across state lines. The revisions to parts 71 and 80 to the Code of Federal Regulation (CFR) regarding the interstate transportation of *Mycobacterium avium* subspecies *paratuberculosis* infected animal need to be expanded and vigorously enforced. Otherwise, the status of the US beef industry may come to resemble the situation in the United Kingdom. In 2008, the number of cattle slaughtered because of bovine tuberculosis in the United Kingdom increased 42% and the number of cattle herds affected increased by 19%. The impending slaughter of over 120,000 dairy cows by the National Milk Producers Federation's herd reduction program, plus the progressive reduction of dairy cows in draught stricken states, will compound the negative effect on beef prices as beef producers begin to

buffer themselves against the potential negative impact of impending quarantine of their herds due to potential infestation by M. bovis.

The growing problems in the dairy and beef industries have the very real potential to undermine public confidence in USDA's policies as it attempts to address the now confirmed issue of *Mycobacterium avium* subspecies *paratuberculosis* being a significant public health hazard. resemble the situation in the United Kingdom. In 2008, the number of cattle slaughtered because of bovine tuberculosis in the United Kingdom increased 42% and the number of cattle herds affected increased by 19%.

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The growing problems in the dairy and beef industries have the very real potential to undermine public confidence in USDA's policies as it attempts to address the now confirmed issue of *Mycobacterium avium* subspecies *paratuberculosis* being a significant public health hazard.

3. List of Recent Publications

Alonso-Hearn M, Eckstein TM, Sommer S, Bermudez LE, 2009. A *Mycobacterium avium* subsp. *paratuberculosis* LuxR regulates cell envelope and virulence. Innate Immun. 2009 Aug 26. [Epub ahead of print].

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