continuing education

Update on paratuberculosis: 3. Control and zoonotic potential

This is the third in the series of papers adapted from the Johne's Information Centre website (http://johnes.org), updated by MICHAEL T. COLLINS, President, International Association for Paratuberculosis.

Introduction

In the first two papers in this series (Collins, 2003a,b) attention was drawn to several features of paratuberculosis in cattle that have implications for the ways in which the disease might be controlled and eliminated from an infected herd. Traditionally thought of as an infection of ruminant animals, the natural host range of *Mycobacterium paratuberculosis* is large and growing. Scottish scientists have convincingly demonstrated *M. paratuberculosis* in rabbits, foxes, stoats, weasels, mice, and voles. The source of infection for these animals is presumed to have been infected domestic livestock. Whether these wild animals are dead-end hosts or have the potential and opportunity to transmit the infection back to domestic animals or simply sustain *M. paratuberculosis* in the ecosystem remains to be determined. The infection appears to be spreading both among and across species. Two reports of spontaneous *M. paratuberculosis* infection in nonhuman primates, stump-tail macques in 1987 and a mandrill baboon in 1994 (Anon, 2003), indicate that these too may be on the list of animals susceptible to *M. paratuberculosis* infection.

There is a considerable literature on the question of the zoonotic potential of *M. paratuberculosis* and it is recognised that if it is ever confirmed that humans acquire disease as a consequence of infection by this bacterium, then the way veterinarians manage the infection in animals will be changed significantly.

In cattle, infection begins in very young animals but the signs of illness do not appear until the host is adult. Infected adults pass the infection to neonates via faeces or milk contaminated with *M. paratuberculosis*. It follows that farm sanitation and management of manure are critical to the control of Johne's disease. The basics of control are simple but, because of the biological characteristics of the organism and the slow onset of pathological changes, a typical herd clean-up programme may take five years or longer.

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- new infections must be prevented;
- infected animals must be identified and removed from the herd ("test and cull");
- the premises must be decontaminated by chemical disinfection or by a fallow interval.

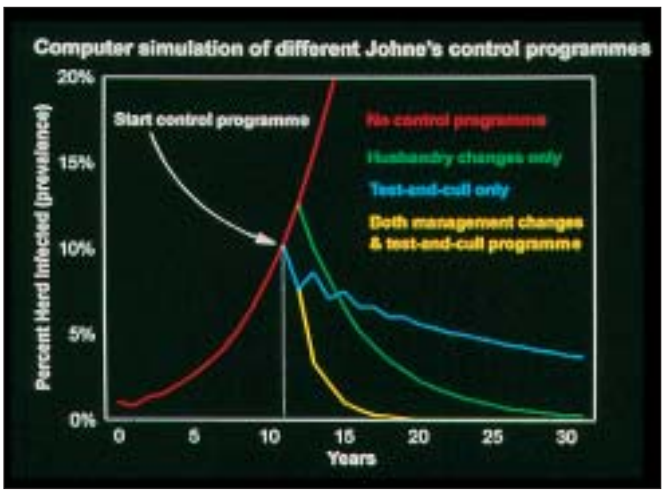

FIGURE 1: A computer simulation model illustrates how control of Johne's disease takes time and how faster control can be accomplished by both changing calf-rearing procedures and testing the adult herd to identify and cull the infectious cows. Of these two basic strategies, changing heifer management to limit the chance of calves becoming infected is the more important.

A computer simulation model (Figure 1) illustrates how control of Johne's disease takes time and how faster control can be accomplished by both changing calf-rearing procedures and testing the adult herd to identify and cull the infectious cows. Of these two basic strategies, changing heifer management to limit the chance of calves becoming infected is the more important.

This paper will describe what is necessary to implement a control programme to eliminate Johne's disease from an infected herd, with particular reference to the dairy herd, and it will conclude with a synoptic overview of current knowledge on the zoonotic potential of *M. paratuberculosis*.

Control measures

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from the herd ("test and cull"); the premises must be decontaminated by chemical disinfection or by a fallow interval.

Prevent new infections in calves

Because young animals are particularly susceptible to infection by *M. paratuberculosis*, it is important to keep them well away from the manure of the adult animals; one or more of which may be excreting the bacterium. Calves should be born in a clean dry environment with minimal faecal contamination. In the infected dairy herd, prompt removal of the newborn from its dam is recommended. This practice may not be practical for other types of cattle enterprise or for other animal species, although in certain circumstances the option to hand-rear and house offspring in a facility separate from adults may have to be considered. The longer the separation of young stock and adults can be maintained the better. For dairy cattle, the minimum time for complete separation is the first six months of life, the "window" of maximum susceptibility.

Cows infected with *M. paratuberculosis* are liable to excrete the bacterium in their milk and it is likely that smaller ruminants like sheep and goats also do so, although such studies have not been reported. Excretion in milk happens most often in animals with clinical signs of Johne's disease, but it can occur in infected animals that appear healthy. Because no diagnostic test can detect all infected animals on a single herd test, it is best if feeding of raw, non-pasteurized milk and natural nursing can be avoided. Artificial milk replacers are pasteurised and considered free of *M. paratuberculosis*. An alternative to milk replacers is the on-farm pasteurisation of waste milk. This method is safe, cost-effective and increasingly employed on large dairies. Both home-made and commercial pasteurisation units are in use on dairies.

Colostrum, which is critical to the health and survival of newborns, can contain *M. paratuberculosis*. The thick viscous nature of colostrum makes it very difficult to pasteurise and so, for practical reasons, it is not advised. The risk of transmitting infection in colostrum can be minimised by

- using colostrum from Johne's test-negative animals only;
- not pooling colostrum from several cows;
- thoroughly cleaning the udder and teats before collection of colostrum to avoid contamination by faeces.

Prevent new infections in older stock

Contamination of feed can occur when the same equipment is used to move both feed and manure - this practice should be avoided. Also, steps should be taken to prevent contamination of water, particularly ponds, wallows or streams from which animals can drink. Theoretically, contamination of pasture with *M. paratuberculosis* is important as a way of transmitting infection but it is less likely than other modes of transmission and is far more difficult to control. If possible, infected pastures should be tilled and re-seeded. When possible, heavily contaminated land should be kept free of animals, especially young animals, while environmental conditions cut down the number of bacteria. The reduction of the bacterial load to a level no longer sufficient to infect an animal can take up to a year or longer, depending on the initial level of contamination.

Removal of the source of *M. paratuberculosis*

Often referred to as a "test-and-cull" programme, this practice is essential to successful control of Johne's disease in herds or flocks in a reasonable period of time. The majority of *M. paratuberculosis* infections in a herd are "invisible". Cows with clinical signs of Johne's disease, diarrhoea and weight loss, are only a small fraction of the truly infected animals. The infection has the ability to silently spread from cows to calves long before signs of illness in infected animals are evident. *M. paratuberculosis* infections can be transmitted from mothers to offspring by contact with the mother's infected faeces, through infected colostrum or milk from the mother, or across the placenta before birth.

Depending on the extent to which the recommendations for management of manure and milk can be implemented, there is a moderate to high probability that calves born to *M. paratuberculosis*-infected mothers will acquire the infection. Consequently, on a case-by-case basis, it may be wise to cull offspring born to infected mothers. If not culled, it may take two or more years to determine if the young animal has become infected, and this will be time lost in pursuit of control or eradication of the *M. paratuberculosis* in the herd.

Culling and replacing productive cows is expensive. An alternative Johne's disease control plan is to label the test-positive cows and manage them accordingly. That is:

- calve them in separate maternity pens,
- do not use their colostrum,
- do not use their milk to feed heifer calves,
- do not bring the most recent crop of their heifer calves into the milking herd.

Whether such a cost-saving programme will still effectively control paratuberculosis has not yet been tested.

Laboratory tests are important to determine which cows are infected. Test-positive cows are generally those most likely to be infectious (excreting *M. paratuberculosis* in milk and manure) and so they should be removed, or at least isolated, from the herd.

Clearly, there are situations where alternatives must be considered: testing and culling of all test-positive animals is not always required. With the great diversity of animal species affected by Johne's disease and the differences in husbandry practices and the economic value of these different species, it is difficult to generalise about test-and-cull recommendations. Decisions on how best to implement testing in a Johne's control programme should be made in consultation with local veterinary officers.

Paratuberculosis in cattle

Despite the many inconsistencies shown by affected animals, the following aide-memoir encapsulates the features of a typical case of Johne's disease.

Aetiology

Mycobacterium paratuberculosis

Pathogenesis

M. paratuberculosis

- ingested by young animal (milk, water, feed);
- adheres to M cells overlying Peyer's patches in terminal ileum;
- invades the lymphoid follicles, is engulfed by macrophages;
- survives and replicates within macrophages;
- evokes release of cytokines that recruit more lymphocytes and macrophages;
- slowly progressive development of granulomatous lesions in ileum;
- can be congenital (transmitted *in utero*).

Pathology

Gross

- hypertrophy of lower small intestine:
- thick mucosal folds ("like convolutions of cerebral cortex"); folds cannot be erased by stretching.
- "cording": of lymphatic vessels on serosal surface and along mesentery to lymph nodes;
- mesenteric lymph nodes enlarged, pale, oedematous.

Microscopic

- infiltration by lymphocytes, plasma cells, eosinophils, macrophages, epithelioid cells, giant cells;
- Ziehl-Neelsen stain: clumps of acid-fast bacilli.

Clinical signs

Do not appear until aged two years plus;

- chronic diarrhoea:
  - profuse, fluid. inoffensive odour, passed without effort;
  - continuous or intermittent.
- bottle jaw (fades as diarrhoea develops);
- loss of production (milk yield often decreased in previous lactation);
- progressive emaciation.

Diagnosis

- faecal smear stained by Ziehl-Neelsen stain;
- culture of faeces;
- serological tests: complement fixation (CFT), agar gel immunodiffusion (AGID), ELISA;
- gamma-interferon test.

Control

- new infections must be prevented;
- infected animals must be identified and removed from the herd ("test and cull");
- the premises must be decontaminated by chemical disinfection or by a fallow interval.

Disinfection

M. paratuberculosis is resistant to most commonly used disinfectants. Destruction of *M. paratuberculosis* on surfaces that might be contaminated requires thorough cleaning with soap and water, followed by application of a disinfectant that is labelled as "tuberculocidal": cresylic disinfectants and sodium orthophenylphenate kill mycobacteria that are not protected by organic matter. Tuberculocidal disinfectants usually contain strong chemical compounds and should be used carefully. The instructions provided on the label for proper use and safe handling should be followed precisely.

Zoonotic potential of *M. paratuberculosis*

Virtually all known mycobacterial pathogens of animals are transmissible to humans and have the capacity to cause disease. Thus, it is plausible that *M. paratuberculosis* might be a potential human pathogen.

The question of the zoonotic potential of *M. paratuberculosis* precedes the question of how humans, most of whom live in cities, could be exposed to this domestic agriculture pathogen, i.e., the route of transmission. If, when, or how *M. paratuberculosis* has the opportunity to infect humans remains a puzzle. However, given the expanding epidemic of paratuberculosis in multiple animal species, it is likely that the burden of *M. paratuberculosis* in the environment is increasing. While questions on transmission are being investigated, they will not be dealt with here as the information is incomplete and currently highly speculative.

The clinical and pathological resemblance of Crohn's disease and Johne's disease was recognised when this chronic inflammatory bowel disease was first recognised in humans in 1913, just 18 years after Johne's disease was first reported. However, as countless subsequent studies failed to identify a mycobacterial agent, the theory of a mycobacterial aetiology fell into disfavour. Diagnostic methods for Johne's disease have steadily improved as this infectious disease has spread in food animals and other ruminants. With new more sophisticated diagnostic tools developed in recent years, the question of whether *M. paratuberculosis* may be involved in Crohn's disease has been re-examined.

The most recent reviews on the *M. paratuberculosis*-Crohn's disease connection can be found in a report of the U.S. National Academies of Sciences (Anon., 2003) and in the August 2003 issue of *The Lancet* (Greenstein, 2003).

Clinically, diarrhoea and weight loss are the predominant signs in both diseases. Abdominal pain and fever are part of the constellation of signs in Crohn's disease but they appear to be absent in Johne's disease.

In terms of pathology, Crohn's disease is a chronic inflammatory bowel disease that most frequently involves the ileum, with an associated colitis in many patients. Diffuse granulomatous inflammation is the hallmark of microscopic findings. The inflammation may occur as a loose collection of macrophages or it may be a more organised lesion containing

multinucleated giant cells, neutrophils, and eosinophils with a small area of central necrosis. The earliest mucosal lesions are pinpoint ulcers, which are thought to relate to the uptake of particulate antigens or organisms by specialised gastrointestinal cells (M cells). Johne's disease also targets the ileum and progresses up and down the gastrointestinal tract, disseminates to regional lymph nodes, and eventually becomes a bacteraemic infection. The primary site of infection is thought to be Peyer's patches with M cells mediating uptake of *M. paratuberculosis*. Host response to infection is primarily that of a cell-mediated inflammatory response leading to transmural, non-caseating granulomatous inflammation with giant cells. Inflammation causes grossly evident thickening of the intestine but ulceration is not a common finding.

The triggering event for Crohn's disease is thought to occur early in life and then be followed by 15 to 30 years incubation or latency period. Johne's disease also has a long interval between infection with *M. paratuberculosis* and onset of clinical signs (two to 10 years). In both diseases, clinical signs are seldom seen before sexual maturity.

A lack of association between Crohn's disease and exposure to animals is often cited as evidence that *M. paratuberculosis* is not the cause of Crohn's disease. (That is, it is thought that the number of people diagnosed with Crohn's disease is no greater among dairy farmers who theoretically work with infected animals than people with no exposure to domestic agriculture species).

One of the more influential facts arguing against involvement of *M. paratuberculosis* in the aetiology of the disease is that special stains for mycobacteria do not reveal the organism in tissues of Crohn's disease patients. Use of new staining techniques changed this. Using genetic probes for *in situ* labelling of cells, Sechi *et al*. (2001)reported that cell wall-deficient *M. paratuberculosis* could be seen in 35 of 48 (73%) of Crohn's patients.

The first reported isolation of *M. paratuberculosis* from Crohn's disease patients was in 1984. Through the 1990s, the bacterium was isolated sporadically but not consistently through culture methods. Reports of sporadic isolation were summarised in a 1996 review article, which described culture of *M. paratuberculosis* from 10 of 135 (7.4%) Crohn's disease patients as compared to one of 121 (0.8%) control subjects. As culture methods improved, rates of isolation of *M. paratuberculosis* increased from both humans and animals. More recently, Bull *et al*. (2003) reported isolation of *M. paratuberculosis* from 14 of 33 (42%) Crohn's patients compared to 3 of 33 (9%) of non-inflammatory bowel disease controls.

When a molecular technique called IS900 PCR probe is used, genetic material from *M. paratuberculosis* is detected in resected bowel tissues from Crohn's disease more consistently than the living organism is isolated by culture. While the detection of the *M. paratuberculosis* genetic material does not imply that the organism is living in the patient, these genetic probe findings suggest an association of *M. paratuberculosis* with Crohn's disease.

Tests for serum antibodies to *M. paratuberculosis* in Crohn's disease patients dating back to the 1980s have reported divergent results; but when purified *M. paratuberculosis* antigens were used in more recent studies, several investigators found a significant association. When Collins *et al*. (2000) used an adaptation of a commercial ELISA for diagnosis of paratuberculosis in cattle they reported that 13.4% of 142 Crohn's disease patients were test-positive for paratuberculosis as compared to 2.6% of healthy blood donors. This difference was statistically significant. Thus far, only one study has reported results of diagnostic assays for cellular immune responses (the cytokine gamma-interferon released after stimulation of leukocytes with antigenic extracts) to *M. paratuberculosis* in Crohn's disease patients. That study found a significant association between a response to *M. paratuberculosis* (hyporesponsiveness) and Crohn's disease (Collins *et al*., 2000).

Clearly, more research is needed before solid scientific conclusions can be made as to whether *M. paratuberculosis* is zoonotic. If it is, it could be an emerging disease problem that must be urgently addressed.

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