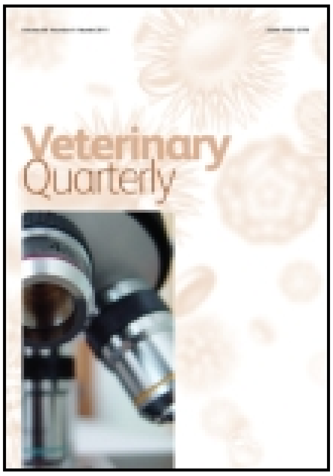

Veterinary Quarterly
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tveq20

Paratuberculosis recognized as a problem at last: A review

D. Bakker [a], P.T.J. Willemsen [b] & F.G. van Zijderveld [b]
[a] Department of Bacteriology, Institute for Animal Science and Health, P.O.Box 65, Lelystad, 8200AB Phone: +31-320-238159 Fax: +31-320-238159 E-mail:
[b] Department of Bacteriology, Institute for Animal Science and Health, P.O.Box 65, Lelystad, 8200AB Phone: +31-320-238159 Fax: +31-320-238159
Published online: 01 Nov 2011.

strate PrP^{BSE} in clinically diseased animals in a 1000 to 2500-fold dilution of brain samples, corresponding to 3.6 - 1.2 μg of tissue equivalents. The preliminary results indicate that the test performs very well in comparison with other tests that have recently been evaluated under the supervision of the EU (7). The test appears to have a detection limit at least as good as these recently evaluated tests and has the additional advantage of being practical and robust.

ACKOWLEDGEMENTS

We thank drs. Van op den Bosch (CODA, Ukkel, Belgium), Weavers (VRL, Dublin, Ireland), Oesch (Prionics, Zürich, Switserland), and Wells (VLA, Weybridge, United Kingdom) for supplying confirmed-positive BSE materials. We thank dr. Mark Rogers (UCD, Dublin, Ireland) for supplying us with recombinant ovine PrP.

REFERENCES

1. Bradley R, and Matthews D. Sub-acute, transmissible spongiform encephalopathies: current concepts and future needs. In: Bradley R, and Matthews D, eds. Transmissible Spongiform Encephalopathies of Animals. Rev sci tech Off int Epiz 1992; 11: 605-34.
2. Braun U. BSE und andere spongiforme Enzephalopathien. Pary Buchverlag, Berlin, 1998.
3. Garssen GJ, Jacobs JG, Smits MA, Keulen LJM van, Schreuder BEC, and Langeveld. JPM. Rapid assay for BSE and scrapie. In: Characterization and Diagnosis of Prion Diseases in Animals and Man, Symposium Tubingen, Germany, 23-25 September 1999.
4. Haritani M, Spencer YI, and Wells GAH. Hydrated autoclave pretreatment enhancement of prion proteinimmunoreactivity in formalin-fixed bovine spongiformencephalopathy-affected brain. Acta Neuropathol Berl 1994; 87: 86-90.
5. Hope J, Reekie LJ, Hunter N, Multhaup G, Beyreuther K, White H, Stack MJ, Dawson M, Wells GAH, and Scott AC. Fibrils from brains of cows with new cattle disease containscrapie-associated protein. Nature 1988; 336: 390-2.
6. Jeffrey M, Halliday WG, and Goodsir CM. A morphometric and immunohistochemical study of the vestibular nuclearcomplex in bovine spongiform encephalopathy. Acta Neuropathol Berl 1992; 84: 651-7.
7. Moynagh J. The evaluation of tests for the diagnosis of transmissible spongiform encephalopathy in bovines. Preliminary Report, DG XXIV, European Commission, Brussels, Belgium, 8 July 1999.
8. Prusiner SB. Prions: novel infectious pathogens. Adv Virus Res 1984; 29: 1-56.
9. Schreuder BEC. General aspects of transmissible spongiform encephalopathies and hypotheses about the agents. Vet Quart 1993; 15: 167-74.
10. Schreuder BEC Animal spongiform encephalopathies--an update. Part II. Bovine spongiform encephalopathy (BSE). Vet Quart 1994; 16: 182-92.
11. Scott AC, Wells GAH, Chaplin MJ, and Dawson M. Bovine spongiform encephalopathy: detection of fibrils in the central nervous system is not affected by autolysis. Res Vet Sci 1992; 52: 332-6.
12. Wells GAH, Hancock RD, Cooley WA, Richards MS, Higgins RJ, and David GP. Bovine spongiform encephalopathy: diagnostic significance of vacuolarchanges in selected nuclei of the medulla oblongata. Vet Rec 1989; 125: 521-4.
13. Wells GAH, Scott AC, Johnson CT, Gunning RF, Hancock RD, Jeffrey M, Bradley R, and Dawson M. A novel progressive spongiform encephalopathy in cattle. Vet Rec 1987; 121: 419-20.
14. Wells GAH, Scott AC, Wilesmith JW, Simmons MM, and Matthews D. Correlation between the results of a histopathological examination and the detection of abnormal brain fibrils in the diagnosis of bovine spongiform encephalopathy. Res Vet Sci 1994; 56: 346-51.
15. Wells GAH, Wilesmith JW, and McGill IS. Bovine Spongiform Encephalopathy:A Neuropathological Perspective. Brain Pathol 1991; 1: 69-78.
16. Wilesmith JW, Hoinville LJ, Ryan JB, and Sayers AR. Bovine spongiform encephalopathy: aspects of the clinical picture and analyses of possible changes 1986-1990. Vet Rec 1992; 130: 197-201.
17. Wilesmith JW, Wells GAH, Cranwell MP, and Ryan JBM. Bovine spongiform encephalopathy: epidemiological studies. Vet Rec 1988; 123: 638-44.

PARATUBERCULOSIS RECOGNIZED AS A PROBLEM AT LAST: A REVIEW

D. Bakker[1,2], P.T.J. Willemsen[1] and, F.G. van Zijderveld[1]

Vet Quart 2000; 22: 200-4

Accepted for publication: July 17, 2000.

SUMMARY

This article attempts to review briefly current opinions on Johne's disease, or paratuberculosis, in ruminants caused by *Mycobacterium avium subsp. paratuberculosis.* Paratuberculosis has been known to be prevalent in domestic livestock, such as cattle, goats, and sheep, for more than a century. Despite this knowledge only minor efforts have been made to control the disease and, with the attention being focussed on the eradication of other diseases, the problem of paratuberculosis has been neglected in most countries in the past decades. However, recent epidemiological surveys performed in Europe showed a high prevalence of paratuberculosis in cattle and sheep, indicating that the situation has become quite alarming. In addition, the possible role of *M. avium subsp. paratuberculosis* in the aetiology of Crohn's disease in humans is still debated, as discussed in this article. Therefore, there is suddenly a renewed interest in paratuberculosis, and the disease is recognized as a significant problem. As a consequence, there is a need for reliable diagnostic tools for large-scale use to allow the introduction of programmes to control and eventually eradicate the disease. The current status and the possibilities for such programmes are discussed.

[1] *Department of Bacteriology, Institute for Animal Science and Health, P.O.Box 65, 8200AB Lelystad. Tel. : +31-320-238159, fax.: +31-320-238153, E-mail: d.bakker@id.wag-ur.nl*

[2] *corresponding author.*

Keywords: paratuberculosis, diagnosis, *Mycobacterium avium subsp. paratuberculosis*, prevalence, Crohn's disease, diagnosis, vaccination, control.

INTRODUCTION

Paratuberculosis in ruminants is characterized by a chronic granulomatous enteritis caused by the slow growing and difficult to culture bacterium *Mycobacterium avium subsp. paratuberculosis*. The chronic enteritis results in malabsorption of essential nutients and accelerated protein loss. Clinical symptoms of the disease are decrease in milk production, progressive weight loss, intractable diarrhoea, wasting, and finally death (for a review see: 45).
In 1895, paratuberculosis was first described in calves as an atypical form of tuberculosis by Johne and Frothingam (21), because of

the presence of acid-fast bacilli. In 1912, Twort and Ingram were able to isolate the causative organism and it was named *Mycobacterium enteriditis chronicae pseudotuberculosae bovis johne* (38). In the following years, the disease became known as Johne's disease, or paratuberculosis, and it was recognized as being caused by *Mycobacterium avium subsp. paratuberculosis*.

The bacterium is a member of the family of mycobacteriaceae and belongs within this family to the *M. avium* complex and shares a high genetic and antigenic homology with the other members of this complex, *M. avium subsp. avium*, and *M. avium subsp. silvaticum*. An important characteristic of *M. avium subsp. paratuberculosis* is its very slow growth, which depends *in vitro* on the presence of the iron-chelating agent, mycobactin. The discovery of the insertion element, IS900, specific *for M. avium subsp. paratuberculosis* has made the detection and discrimination of this organism easier (16).

M. avium subsp. paratuberculosis can infect a wide range of ruminants, and also wild rabbits (17), foxes, weasels, and ferrets (2), but it is particularly prevalent in dairy herds and other domestic livestock such as sheep, goats and farmed deer.

The major source of infection is faeces excreted by infected animals. Under natural conditions, the disease in cattle spreads by ingestion of *Mycobacterium avium subsp. paratuberculosis* from the contaminated environment. Shedding can be particularly high (more than 10^8 mycobacteria per gram of faeces) during the clinical stage of the disease. Faecal contamination of teats and the presence of mycobacteria in colostrum and (tank-)milk may cause sucking neonatal animals to ingest large doses of the organism; contaminated pasture, water and feed may also be responsible for infection (8). The fact that the bacterium is able to survive for more than a year in the environment makes it difficult to stop transmission within a herd without extensive management changes (24).

M. avium subsp. paratuberculosis has also been isolated from the uterus and placenta of infected cows, and intra-uterine infection of the foetus can occur. Susceptibility to infection is highest in animals younger than 30 days, but clinical disease does not usually develop in dairy cattle until 2-5 years of age. The establishment of the infection depends on the number of bacteria ingested. During this long incubation period or subclinical stage, the animal starts shedding low numbers of bacteria and will infect other animals before clinical signs become apparent and the animal is removed from the herd (36). The onset of clinical disease is unpredictable, but may be triggered by stress caused by factors such as parturition, low nutrition, transport, high milk production, introduction to a new herd, and concurrent illness.

Paratuberculosis in a herd will result in considerable economic losses to the herd owner. These losses are mainly caused by a decreased milk production and early replacement of the animals (32). Benedictus *et al.* (3) calculated that, in addition to the more obvious costs, such as reduced slaughter value and production losses in the clinical stage of the disease, paratuberculosis can result in significant production losses in the subclinical stage. In modern dairy farming, the decrease in milk production will result in an early removal of the animal from the herd without the problems being attributed to an infection with *M. avium subsp. paratuberculosis.*

Despite the fact that the disease has been known for more than a century and that it causes considerable economic loss, the control and eradication of paratuberculosis has received relatively little attention. For some countries where bovine tuberculosis is still endemic, it is not regarded to be a priority, whereas in other countries (e.g. the Netherlands) early attempts to control the disease have failed (4). However, in the last few years more attention to paratuberculosis has been paid as a result of a combination of several factors: an apparent increase in the prevalence of paratuberculosis, confirmed by epidemiological surveys in several European countries, and continuing speculation about a possible role for *M. avium subsp. paratuberculosis* in Crohn's disease in humans. In the following sections we attempt to deal with several of these aspects and discuss problems encountered in diagnosing the disease and possibilities for programmes to control paratuberculosis.

A ROLE IN THE AETIOLOGY OF CROHN'S DISEASE?

Crohn's disease was first described by the Scottish surgeon Dalziel in 1913 (11) as a chronic regional ileitis. Its current name is derived from an article in 1932 by Crohn *et al.* (10). Crohn's disease belongs with ulcerative colitis to the group of chronic inflammatory bowel diseases. Because of the granulomatous aspects of the disease, Crohn's disease had been confused with intestinal tuberculosis, a disease not uncommon in the days before the pasteurization of milk (6). However, there are characteristic differences between the two diseases: in Crohn's disease, acid-fast bacilli can not be cultured from or visualized by staining of affected tissues. Because of the unknown aetiology of the disease, numerous viruses and bacteria have been examined and named as possible causative agents of the disease in the past 70 years (37). Isolation of *Mycobacterium avium subsp. paratuberculosis* from tissues of Crohn's disease patients in 1984 by Chiodini (9) in the U.S., followed by others, including Haagsma in the Netherlands (18), marked the beginning of speculation about a possible role for this bacterium in the aetiology of Crohn's disease.

In the light of this, 15 years later still continuing, speculation, several European Union member states asked the Scientific Committee on Animal Health and Animal Welfare for an opinion on this issue. The main conclusion of their report (12) is that 'the currently available evidence is insufficient to confirm or to disprove that *M. avium subsp. paratuberculosis* is a causative agent of at least some cases of Crohn's disease in man'. Increased research is recommended both in the area of Crohn's disease and in the area of paratuberculosis, in order to gain a better insight into the multifactorial aspects likely to be involved in the aetiology of Crohn's disease and to develop tools, such as efficient tests, that would enable and encourage programmes for eradication of the disease.

PREVALENCE OF PARATUBERCULOSIS

Even though paratuberculosis is present world-wide, its actual prevalence in most countries is unknown. This is caused by both the low priority of the problem, because in many countries concern about other diseases prevails, and the difficulty to detect infected animals accurately. Nevertheless, data are available for several countries based on bacteriological surveys at slaughterhouses and, more recently, based on antibody detection in serum-samples or bulk-tank milk samples. In slaughterhouse surveys in Denmark, Jørgensen (22) found 2.3% of the adult cattle to be culture positive in 1965. In an identical study performed in 1972, this number had risen to 9.8% (23). Recent studies based on serological testing performed in the Basque country in Spain found 67% of the cattle herds (25) and 32% of the sheep flocks (26) to be positive. Recently, in Belgium the seroprevalence was found to be 17.4% at the herd level and 1.2% at the animal level (5). In the Netherlands, the seroprevalence was estimated to be 54.7% at the herd level and 2.5% at the animal level(30). Given the uncertainties in the performance with regard to sensitivity and specificity of the tests used, the true prevalence in the Dutch study was estimated to be between 30% and 70% at the herd level and between 2.7% and 6.9% at the animal level. In Denmark, a tank-milk ELISA was used to estimate the prevalence of paratuberculosis (31). Samples were taken from 900 herds and 70% were classified as positive, indicating that paratuberculosis in Denmark is also more widespread than previously thought. So far, based on several surveys, Sweden is the only country claiming to be free of paratuberculosis (41). Despite the fact that in most studies different methods were used, which makes comparison of the results difficult, the results indicate that the prevalence of paratuberculosis in domestic livestock is widespread.

DIAGNOSIS OF PARATUBERCULOSIS

In paratuberculosis, as in other mycobacterial infections (e.g. leprosy) an immunological spectrum exists (7). The type of immune response depends on the individual animal, but there is usually a cell-mediated immune response and a low humoral response in the beginning, the tuberculoid form, and as the disease progresses, the situation reverses: there is a stronger humoral response initiated by the release of bacteria by dying macrophages, the lepromatous form of the disease. This is of great importance because the usability of the diagnostic tests will depend on the type of immune response raised by the individual animal and/or the number of bacteria shed in the faeces by this animal.

The available diagnostic tests can be divided into two groups. The first group of tests is aimed at the detection of *M. avium subsp. paratuberculosis*. These tests have high specificity and, since the number of bacteria present increases, the sensitivity increases as the disease progresses. These tests are microscopic examination of faecal smears, culture of the organism from faecal samples, and detection by polymerase chain reaction (PCR).

The second group consists of the immunological tests. These are the enzyme-linked immunosorbent assay (ELISA), using a variety of different antigens, the complement fixation test (CFT), the agar-gel-immuno diffusion test (AGID), the delayed type hypersensitivity test or intradermal test, and the gamma-interferon test.

Conventional bacteriological culture of *M. avium subsp. paratuberculosis* from faecal samples is, certainly in advanced stages of the disease, the most sensitive of these tests, 100 % specific and therefore regarded as the 'gold standard'. Its 'true' sensitivity, meaning the ability to detect infected animals at any one time point, is difficult to assess. This can only be done by following animals in an infected herd for a longer period of time. A sensitivity of approximately 35% has been estimated (44).

Because of the very slow growth of the organism, cultural examination can take up to 6 months to complete and thus it is crucial to eliminate other fast-growing bacteria and fungi from the samples. Since mycobacteria possess a very thick lipid-rich and acid-resistant cell wall, decontamination can be performed by treatment of the sample with acids such as oxalic acid or chemicals such as hexadecylpyridinium (43). These decontamination procedures cause the test to be very labour intensive, time consuming, expensive, and this limits the number of samples that can be handled. Nevertheless, quite substantial losses of samples occur due to overgrowth by fungi or bacteria. In addition, the long culture period prevents rapid action: detecting a positive sample means that a cow has been shedding the bacterium, and thus infecting other animals, for at least several months before the animal is removed from the herd. This limits the use of this test in a certification programme.

The PCR reaction could offer an attractive alternative for the cultural methods: it is rapid, there are no problems with overgrowth by other micro-organisms, and when the right set of primers is used, the PCR is very specific (46). Despite these advantages, implementation of this technique as a routine diagnostic tool for large-scale usage has not been successful thusfar. The main drawback of the technique is the elaborate sample preparation procedures needed to remove inhibitors of the PCR reaction, which are present in faeces and which reduce the sensitivity of the test enormously. As a result, the number of samples that can be processed is greatly reduced and this makes the technique (at present) even more expensive than traditional cultural methods.

The convenience of sample collection, large capacity, rapid laboratory turnaround time, and low cost make a serological test in an ELISA format the preferred test for the large-scale testing needed for a certification and/or eradication programme. However, faecal culture and antibody ELISA's are only suitable for use as tests on older animals because the antibody response increases at a late stage of the disease. Therefore, the ELISA will have a sensitivity rising from zero at early stages of disease to 100% when the animal starts to show clinical symptoms.

Thus far, the detection of serum antibodies against *M. avium subsp. paratuberculosis* is achieved using different crude fractions of the bacterium as antigen. However, false-positive reactions occur as a result of the presence of related bacteria in the environment, e.g. different mycobacterial species can be cultured from soil and from water supplies. Therefore the specificity of the ELISA is enhanced by pre-absorbing the serum sample with crude fractions from another mycobacterium, *M. phlei*, to remove cross-reacting antibodies (47). Since these absorbed ELISAs have become commercially available, they have become the most widely used serological test for paratuberculosis in cattle.

For several years, the absorbed ELISAs were advocated to be the only tests needed for a certification programme. However, the reported relative sensitivity of the absorbed ELISAs is gradually decreasing, from 57% (29) to 33% (44), meaning the ELISA will detect only a third of the animals found positive in concurrent faecal culture.

Diagnostic tests aimed at the detection of an early (T-cell-mediated) immune response, e.g. the skin test and the gamma-interferon assay, could potentially be attractive additions to the array of diagnostic tools available for the detection of paratuberculosis. However, the antigen routinely used for these tests, Johnin PPD, produces non-specific reactions in young animals (33). Therefore, the diagnosis of paratuberculosis would greatly benefit from the identification of more specific antigens for paratuberculosis. Despite claims made previously by several research groups, such antigens are not known at present (12).

The low sensitivity of the available diagnostic tests, especially tests suitable for the detection of young animals in early stages of infection, indicates that it will be very difficult to achieve control and eradication of paratuberculosis in the near future.

VACCINATION AGAINST PARATUBERCULOSIS

Vaccination against paratuberculosis was first introduced in 1926 by Vallee and Ringard (39). The vaccination was performed by the subcutaneous injection of living, unattenuated, *M. avium subsp. paratuberculosis*. Since then, many different formulations of the vaccine have been evaluated, using live bacteria of both unattenuated and attenuated strains, heat-killed organisms as well as disrupted fragments of *M. paratuberculosis*. Routinely, the vaccine is administered within 30 days of birth, subcutaneously in the brisket, where the vaccinal nodule, an undesirable side-effect of vaccination, will be less obtrusive. Even though clinical disease does occur in the first years after the start of vaccination on a farm (42), in general, vaccination will not only reduce the number of animals with clinical disease, but also the number of excretors and the level of excretion (28). However, a Dutch report states that vaccination does not reduce the overall prevalence of infection in a herd (40).

Vaccination alone is not sufficient to control paratuberculosis: husbandry and control measures are needed to obtain maximum benefits (1). Since these changes in management coincide with the start of a vaccination programme, the efficacy of the vaccination itself is often difficult to assess.

Vaccinated animals will respond positively to routine immunological diagnostic tests for paratuberculosis (34,35). Thus, faecal culture remains the only available test to recognize the remaining infected animals. Since the reduction in the level of excretion makes it more difficult to recognize the remaining carriers, vaccinates must not be regarded as free of paratuberculosis and vaccinated herds should be treated as infected herds.

This sensitization not only affects the paratuberculosis diagnostics, but vaccinated animals also become sensitized to bovine PPD used in the intradermal test in control and eradication programmes of bovine tuberculosis (20). Comparative skin tests using bovine PPD and Johnin can be used to differentiate between reactions to *M. bovis* and reactions caused by the vaccination (13,20). However, it was shown that in some cases the bovine reaction was larger than the avian reaction, thus leading to false-positive reactions (19). This limits the large-scale use of the available vaccines particularly in regions where bovine tuberculosis is endemic.

In several countries, vaccination has been used to control the disease. Especially, for controlling paratuberculosis in small ruminants, vaccination is often the only affordable option and has been shown to be quite successful (15).

The adoption of a vaccination programme should be considered in heavily infected herds, when the practitioner considers that control will not be achieved by husbandry and management changes alone. This will prevent considerable losses and aid in the economical survival of the farmer(14).

FUTURE PROSPECTS: CONTROLLING PARATUBERCULOSIS

The high prevalence of paratuberculosis, the fact that it seems to be increasing, and the significant economic losses the disease causes, make it clear that there is a need for the introduction of control (and eventually eradication) programmes.

Any conceivable control programme will have to consist of a combination of strict herd management measures and extensive testing to detect infected animals. Husbandry measures have two objectives: first, to prevent further spread within the infected herd, and second, to prevent the introduction of the infection into an uninfected herd. Important measures will be to keep a closed herd, to stop the feeding of calves with mixed colostrum and the feeding of tank-milk without pasteurisation, and to introduce separate (and clean) calving pens and separate pastures to keep the young animals away from the older animals, etc.

The nature of the disease and the absence of the 'perfect test' make it impossible to detect, at a given time point, all infected animals and to remove them from the herd. Especially infected young animals can easily escape detection. The fact that different infected animals within a herd are likely to be in a different stage of infection and will therefore respond to different tests complicates the diagnostics greatly. However, even though the tests are not suitable for certifying individual animals, they can be used for certifying the paratuberculosis status of the whole herd. In addition, repetitive testing will give, with each round of testing, more guarantees about the status of the herd. Since the most widely used tests, the absorbed ELISA and faecal culture, are most suitable for older animals, the cost of such a certification scheme can be greatly reduced by testing older animals only. Given the low sensitivity of the available tests, numerous rounds of testing will be needed. Recent experience in the Netherlands (using faecal culture as a diagnostic tool) showed that, even when herds with no known history of paratuberculosis were tested, positive herds could still be identified after five rounds of testing at 6 month intervals (45). This means that after several years of investments, these herds were labelled as infected. Experiences like these illustrate why, at present, there is less optimism about the feasibility of rapidly controlling paratuberculosis, without the prior development of improved diagnostic tools, the characterization of more specific antigens to be used for the detection of antibodies or T-cell response, and an efficient vaccine with accompanying diagnostic test to distiguish infected animals from non-infected, vaccinated animals.

REFERENCES

1. Argente G. Efficiency of vaccination and other control measures estimated by fecal culturing in a regional program. In : Proceedings of the Third Collloquium on Paratuberculosis. Eds Chiodini RJ, and Kreeger JM. 1991: 495-8.
2. Beard PM, Henderson D, Daniels M, Pirie A, Buxton D, Hutchings MR, McKendrick I, Rhind S, Stevenson K, and Sharp M. Evidence for paratuberculosis in fox (Vulpes vulpes) and stoat (Mustela erminea). Vet Rec 1999; 145: 612-3.
3. Benedictus G, Dijkhuizen AA, and Stelwagen J. Economic losses due to paratuberculosis in dairy cattle. Vet Rec 1987; 121 (7): 142-6.
4. Benedictus G. Evaluation of organised control of bovine paratuberculosis in Friesland province. Tijdschr Diergeneeskd 1984; 109: 905-16.
5. Boelaert F, Walravens K, Vermeersch JP, Raskin A, Dufey J, and Godfroid J. Sample survey for estimating the herd and individual animal seroprevalence for bovine paratuberculosis in Belgium. (submitted Vet Microbiol)
6. Chiodini RJ. Historical overview and current approaches in determining a mycobacterial etiology of Crohn's disease. In: Is Cohn's disease a mycobacterial disease? Eds Mulder CJJ and Tytgat GNJ Kluwer Academic Publishers. 1992: 1-15.
7. Chiodini RJ. Immunology: resistance to paratuberculosis. Vet Clin Of North Am (Food Animal Practice) 1996; 12 (2): 313-43.
8. Chiodini RJ, Kruiningen HJ van, and Merkal RS. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet 1984; 74 : 218-62.
9. Chiodini RJ, Kruiningen HJV, Merkal RS, Thayer WR, and Coutu JA. Characteristics of an unclassified *Mycobacterium* species isolated from patients with Crohn's disease. J Gen Microbiol 1984; 20: 966-71.
10. Crohn BB, Ginzburg I, and Oppenheimer GD. Regional ileitis: a pathologic and clinical entity. J Am Med Assoc 1932; 99:1323-8.
11. Dalziel TK. Chronic intestinal enteritis. British Med J 1913; 2:1068-1070.
12. European Commission Directorate-General Health and Consumer Protection. 2000; Possible links between Crohn's disease and paratuberculosis. Report of the Scientific Committee on Animal Health and Animal Welfare.
13. Doyle TM. Vaccination against Johne's disease. Brit Vet J 1960; 116: 294-301.
14. Dijkhuizen AA, Schaik G van, Huirne RBM, Kalis CHJ, and Benedictus G. A cost benefit analysis of vaccination against paratuberculosis in dairy cattle. The Kenya Veterinarian 1994; 18: 219-21.
15. Garcia Marin JF, Tellechea J, Gutierrez M, Corpa JM, and Perez V. Evaluation of two vaccines (killed and attenuated) against small ruminants paratuberculosis. In: Proceedings of the Sixth International Colloquium on Paratuberculosis. Eds Manning EJB, and Collins MT. 2000: 234-41.
16. Green EP, Tizard MLY, Moss MT, Thompson J, Winterbourne DJ, McFadden JJ, and Hermon-Taylor J. Sequence and characteristics of IS900 an insertion element identified in a human Crohn's disease isolate of *Mycobacterium paratuberculosis*. Nucleic Acids Research 1989; 17 9063-73.
17. Greig A, Stevenson K, Pérez V, Pirie AA, Grant JM, and Sharp JM. Paratuberculosis in wild rabbits (Oryctolagus cuniculus). Vet Rec 1997; 140: 141-3.
18. Haagsma J, Mulder CJJ, Eger A, and Tytgat GNJ. In: Is Crohn's disease a mycobacterial disease? 1992; Pp16-21. Eds Mulder CJJ, and Tytgat GNJ. Kluwer Academic Publishers.
19. Herbert CN, Doyle TM, and Paterson AB. Tuberculin sensitivity in attested cattle vaccinated against Johne's disease. Vet Rec 1959; 71: 108-11.
20. Ingliss JSS, and Weipers M. The effect of Johne's vaccination on the efficiency of the single intradermal comparative tuberculin test. Brit Vet J 1963; 119: 426-9.
21. Johne HA, and Frothingam L. Ein eigenthümlicher Fall von Tuberculose beim Rind. Deutsch Zeitschr Tiermed Vergl Path 1895; 21:438-54.
22. Jorgensen JB. On the occurrence of Mycobacterium johnei in the mesenteric lymphnodes of abattoir cattle. Nord Vet Med 1965; 17: 97-102.
23. Jorgensen J B. Studies on the occurrence of paratuberculosis in Denmark. Nord Vet Med 1972; 24: 297-308.
24. Jorgensen JB. Survival of *Mycobacterium paratuberculosis* in slurry. Nord Vet Med 1977; 29 (6): 267-70.
25. Juste RA, Garrido J, and Aduriz G. La paratuberculosis desde Neiker IV reunion sobre paratuberculosis en Espana. Derio Spain October 26-27 1999.
26. Juste RA, III reunion sobre paratuberculosis en Espana. Informes Technicos 34 Dpto De Agricultura Y Pesca Gobierno Vasco 1990.
27. Kalis CHJ, Barkema HW, and Hesselink JW. Herd certification for paratuberculosis in unsuspected dairy herds using cultures of strategically pooled faecal samples. In: Proceedings of the Sixth International Colloquium on Paratuberculosis. Eds Manning EJB and Collins MT. 2000: 55-8.
28. Kormendy B. The effect of vaccination on the prevalence in large dairy herds. Vet Microbiol 1994; 41: 117-25.
29. Milner AR, Mack WN, Coates KJ, Hill J, Gill I, and Sheldrick P. The sen-

sitivity and specificty of a modified ELISA for the diagnosis of Johne's disease from a field trial in cattle. Vet Microbiol 1990; 25: 193-8.
30. Muskens J, Barkema HW, Russchen E, van Maanen K, Schukken YH, and Bakker D. Prevalence and regional distribution of bovine paratuberculosis in dairy herds in the Netherlands, (submitted Vet Microbiol).
31. Nielsen SS, Thamsborg SM. Houe H, and Bitch V. Bulk-tank milk ELISA antibodies for estimating the prevalence of paratuberculosis in Danish dairy herds. Prev Vet Med 2000; 44: 1-7.
32. Ott SL, Wells SJ, and Wagner BA. Herd level economic losses associated with Johne's disease on US dairy operations. Prev Vet Med 1999; 40: 179-92.
33. Stabel J. Production of gamma-interferon by peripheral blood mononuclear cells: an important diagnostic tool for the detection of subclinical paratuberculosis. J Vet Diagn Invest 1996; 8: 345-50.
34. Spangler E, Heider LE, Bech-Nielsen S, and Dorn CR. Serologic ELISA responses of calves vaccinated with a killed M.paratuberculosis vaccine. Am J Vet Res 1991; 52: 1197-1200.
35. Stuart P, Vaccination against Johne's disease in cattle exposed to experimental infection. Brit Vet J 1965; 121: 289-318.
36. Sweeney RW. Transmission of paratuberculosis. Vet Clin Of North Am (Food Animal Practice) 1996; 12 (2): 305-12.
37. Thompson DE. The role of mycobacteria in Crohn's disease. J Med Microbiol 1994; 41: 74-94.
38. Twort FW, and Ingram GLY. A method for isolating and cultivating the *Mycobacterium enteritides chronicae pseudotuberculosae bovis* Johne and some experiments on the preparation of a diagnostic vaccine for pseudotuberculous enteritis of bovines. Vet J 1912; 68: 353-65.
39. Vallee H, and Rinjard P. Etudes sur l'enterite paratuberculeuse des bovides (note preliminaire). Rev Gen Med Vet 1926; 35: 1-9.
40. Van Weering HJ, Breukink H, Giessen J van der, Haagsma J, Kalis CHJ, Lange D de, Snoep JJ, Vries G de, and Wentink GH. Intermediate report on the vaccination of Johne's disease in the Netherlands. The Paratuberculosis Newsletter 1995; 7: 4-8.
41. Viske D, Larsson B, Engvall A, and Bölske G. Paratuberculosis in Sweden. Epidemiologie et Sante Animale 1997; 31: 31-2.
42. Wentink GH, Bongers JH, Zeeuwen AAPA, and Jaartsveld FHJ. Incidence of paratuberculosis after vaccination against *Mparatuberculosis* in two infected dairy herds. J Vet MedB 1994; 41: 517-22.
43. Whipple DL, Callihan DR, and Jarnigan JL. Cultivation of *Mycobacterium paratuberculosis* from bovine fecal specimens and a suggested standardized procedure. J Vet Diagn Invest 1991; 3 (4) : 368-74.
44. Whitlock RH, Wells SJ, Sweeney RW, and Tiem J van. ELISA and fecal culture: sensitivity and specificity for each method In: Proceedings of the Sixth International Colloquium on Paratuberculosis. Eds Manning EJB and Collins MT. 2000: 353-62.
45. Whitlock RH, and Buergelt C. Preclinical and clinical manifestations of paratuberculosis. Vet Clin Of North Am (Food Animal Practice) 1996; 12 (2): 345-56.
46. Willemsen PTJ, Bakker D, Damman M, Eger A, and van Zijderveld FG. An error in the reported IS900 nucleotide sequence affects the proposed expression of ORF2 (Hed) and the detection of M.a.paratuberculosis using the polymerase chain reaction. In: Proceedings of the Sixth International Colloquium on Paratuberculosis. Eds Manning EJB and Collins MT. 2000: 466-71.
47. Yokomizo Y, Kishima M, Mori Y, and Nishimori K. Evaluation of enzyme-linked immunosorbent assay in comparison with complement fixation test for the diagnosis of subclinical paratuberculosis in cattle. J Vet Med Sci 1991; 53 (4): 577-84.

DEFENCE MECHANISMS AGAINST VIRAL INFECTION IN POULTRY: A REVIEW

S.H.M. Jeurissen[1,3], A.G. Boonstra-Blom[1], S.O. Al-Garib[2], L. Hartog[2], and G. Koch[2]

Vet Quart 2000; 22: 204-8
Accepted for publication: July 11, 2000

SUMMARY

Defence against viral infections in poultry consists of innate and adaptive mechanisms. The innate defence is mainly formed by natural killer cells, granulocytes, and macrophages and their secreted products, such as nitric oxide and various cytokines. The innate defence is of crucial importance early in viral infections. Natural killer cell activity can be routinely determined in chickens of 4 weeks and older using the RP9 tumour cell line. In vitro assays to determine the phagocytosis and killing activity of granulocytes and macrophages towards bacteria have been developed for chickens, but they have not been used with respect to virally infected animals. Cytokines, such as interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α, are indicators of macrophage activity during viral infections, and assays to measure IL-1 and IL-6 have been applied to chicken-derived materials.

The adaptive defence can be divided into humoral and cellular immunity and both take time to develop and thus are more important later on during viral infections. Various enzyme-linked immunosorbent assays (ELISAs) to measure humoral immunity specific for the viruses that most commonly infect poultry in the field are now commercially available. These ELISAs are based on a coating of a certain virus on the plate. After incubation with chicken sera, the bound virus-specific antibodies are recognized by conjugates specific for chicken IgM and IgG. Cytotoxic T lymphocyte activity can be measured using a recently developed in vitro assay based on reticuloendotheliosis virus-transformed target cells that are loaded with viral antigens, e.g. Newcastle disease virus. This assay is still in an experimental stage, but will offer great opportunities in the near future for research into the cellular defence mechanisms during viral infections.

1 *Department of Immunology, Pathobiology and Epidemiology, Institute for Animal Science and Health ID-Lelystad, Lelystad, the Netherlands.*
2 *Department of Avian Virology, Institute for Animal Science and Health ID-Lelystad, Lelystad, the Netherlands.*
3 *Correspondence to : Dr. S.H.M. Jeurissen, Institute for Animal Science and Health ID-Lelystad, P.O. Box 65, 8200 AB Lelystad, the Netherlands, tel: +31 320.238238, fax: +31.320.238050, E-mail: s.h.m.jeurissen@id.wag-ur.nl*

INTRODUCTION

World-wide, the consumption of poultry products is still increasing: in 1998 the production of eggs was 48 million tons and the production of chicken meat exceeded 51 million tonnes (27). The growing number of commercial flocks increases the number of birds at risk of getting infected with pathogens, such as bacteria, viruses and parasites. In view of the economic importance of poultry, much effort has been put into protecting chickens from being infected by improving their innate and adaptive defence, for instance, by adjusting feed and management condi-