

**Abstracts from Oral and Poster presentations
at the**

**Sixth International Colloquium
on Paratuberculosis**

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Title Progress in national control and assurance programs for bovine Johne's disease in Australia.

Author(s) Kennedy DJ, Allworth MB.

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Abstract Cattle strains of M paratuberculosis are known to infect cattle, goats and alpaca in south-eastern Australia, where there are also significant numbers of farmed deer. Epidemiological evidence in Australia to date supports the distinction between bovine Johne's disease (BJD) caused by cattle strains and ovine JD (OJD), caused by sheep strains in sheep and goats. The National JD Control Program is coordinated by the Australian Animal Health Council Ltd (AAHC), working with the livestock industries and with the Commonwealth, State and Territory governments. The Council also brokers industry and government funding for the program. The National Johne's Disease Market Assurance Program for cattle was launched in 1996 as the first of a suite of voluntary national market assurance programs (MAPs) to assess and certify herds as negative for JD. In the following two years, 450 herds achieved an assessed status. MAPs were also developed for alpaca and goats in 1998 (and for sheep in 1997). National standards for state control of JD through zoning, movement controls and procedures in infected and suspect herds have also been developed. National standards and programs have been reviewed and improved. The paper will cover factors affecting development and implementation, uptake of and improvements to national control and assurance programs in Australia.

Title Progress in national control and assurance programs for Ovine Johne's disease in Australia.

Author(s) Allworth MB, Kennedy DJ.

Institution "Talooby" Holbrook NSW 2644, Australia.

Abstract Ovine Johne's Disease (OJD) was first diagnosed in Australia in 1980. Since then, a gradual increase in the number of identified infected flocks has occurred, with 36 flocks detected by 1991, and 192 flocks by 1996. The first cases were detected in central NSW, but since 1995, OJD has been diagnosed in Victoria, Flinders Island (Tasmania) and South Australia. There are currently 335 known infected flocks. A National control program has been introduced. Initially, this has involved the development of a Market Assurance Program, and the agreement between States on Standard Definitions and Rules. Concurrently, States initiated their own control programs. The major issue has been whether or not to embark on a national eradication program. Debate has mainly centred on whether there is a 'window of opportunity' before the disease becomes widespread, or whether it has already spread to too many flocks. An interim Surveillance and Research Program, costing \$2.45m, was initiated while Industry and Governments developed a six year Research and Combative Program to control and further evaluate the feasibility of eradication and alternative control methods. The paper will outline the current aspects of the National OJD Program, including the recently endorsed six year business plan. Issues central to the "OJD debate" will also be addressed.

Title U.S. Voluntary Johne's Disease Herd Status Program for Cattle.

Author(s) Bulaga LL¹, Collins MT².

Institution USAHA National Johne's Working Group Herd Certification Subcommittee. ¹ USDA APHIS Veterinary Services, Robbinsville New Jersey, USA. ² School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin, USA.

Abstract Johne's disease occurs in cattle herds throughout the United States. A recent national survey found 22 percent of dairy herds are infected. Results from a national study of beef cattle are pending. In 1993 the United States Animal Health Association (USAHA) adopted a model Johne's disease herd certification program. However, few herd owners elected to pursue certification, citing the amount of testing and associated cost as a hindrance. In 1997, USAHA

National Johne's Working Group appointed a committee to design a more affordable, flexible and yet scientifically sound herd certification program. The result is the U.S. Voluntary Johne's Disease Herd Status Program (VJDHSP). The VJDHSP provides minimal requirements for a program to identify herds of low risk of *M. paratuberculosis* infection. The program consists of two tracks - Standard and Fast Track - with four Levels. The Standard Track begins with a minimal financial investment and requires at least three years and four tests to reach Level 4. Fast Track herds skip Level 1 with a statement by the herd owner that Johne's disease is not known or suspected to have existed in the herd of origin during the past five years. Fast Track herds may reach Level 4 in two years with three tests. Both tracks utilise various combinations of ELISA testing and faecal culture over time. The U.S. VJDHSP is part of a national strategic plan, which includes a national educational campaign and guidelines for states to assist infected herds. The strategic plan is designed to reduce the prevalence and eventually eradicate Johne's disease from the United States.

Title Progress in the US Approach to Johne's Disease Control on the Farm.

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Abstract The effort to control of Johne's disease in the US has gained momentum among States, national industry organizations, and veterinarians in the past two years. Driving factors have been a national dairy survey (NAHMS '96), commitments by national cattle and beef industry organizations to educate their industry about Johne's disease, and awareness of the association with human Crohn's disease. Results of NAHMS '96 were widely disseminated by industry and scientific articles and indicated that 20-40% of herds were likely infected with Johne's disease and that 50% of owners had little knowledge. The US has adopted an education first strategy with states and industry being expected to take the lead role. USDA APHIS does not fund Johne's disease but supports the development of voluntary industry driven programs. As of fall 1998, National Guidelines to identify test negative status herds have been developed and several States have instituted some type of program to aid control on farms. Three examples of US efforts to promote control of Johne's disease will be presented. The first is a Johne's Disease Prevention and Control Measures Manual for Cattle Producers produced by the National Johne's Disease Working Group, subcommittee of the USAHA. The manual contains information about Johne's disease, control, developing specific farm plans, test and interpretation, and worksheets to guide development of control plans for dairy and beef operations. First Edition materials are being distributed through industry organizations and the USDA APHIS Johne's web site. Second is the New York Cattle Health Assurance Program (NYSCHAP). This is a voluntary, flexible, tailored-to-the-farm program initiated by New York State in 1997. The objective is to encourage producers and veterinarians to engage in establishing "best management practices" (BMPs), accepted practices that help maintain animal health and have potential to enhance the quality of products leaving the farm. Producers can participate by addressing specific disease issues with a management plan. A herd health audit approach is used for Johne's disease, to identify a farm team, review farm goals and performance, do a risk assessment, and develop a herd plan. Producers can participate at three levels, at their pace: Participating (BMPs), Control (BMPs plus testing), or Test Negative. Third is a Johne's Disease Control Planning Checklist and Herd Testing Strategy Planner. This is a laminated 2-page on-farm tool designed as a "prompter" developed at Cornell to guide veterinarians step by step through assessing the extent, impact and risks of Johne's in client's herds and customizing a management and herd testing strategy for control. It is available for use and evaluation in the field by veterinarians.

- Title** Herd certification for paratuberculosis in unsuspected dairy herds using cultures of strategically pooled faecal samples.
- Author(s)** Kalis CHJ, Barkema HW, Hesselink JW.
- Institution** Department of Ruminant Health, Animal Health Service, PO Box 361, 9200 AJ Drachten, The Netherlands.
- Abstract** In 1995 the objective was formulated to stop the spread of paratuberculosis in dairy herds in the three northern provinces of The Netherlands. This objective was striven after by formation of a group of dairy herds unsuspected from paratuberculosis, thus providing safe replacement animals to others. Selection of the herds was based on two criteria: (1) absence of clinical paratuberculosis in the last five years, declared by both herdsman and veterinarian and (2) closed herd management since three years. Strategic pooled faecal culture of all adult cows (>24 months) was performed at six months intervals. Hundred and thirteen herds were selected. Herd size varied from 20 to 280 adult dairy cows. The first examination was a check of the laboratory records of the Dutch Animal Health Service. Thirteen herds were suspected of paratuberculosis because of registered positive test results from individual blood or faecal samples in the preceding 5 years. This was in contradiction with the farmer- and veterinarian declaration. The hundred remaining herds entered the culture program. From these 100 herds respectively 13, 8, 10 and 9 herds were removed from the program because of culture of Mycobacterium paratuberculosis (Mptb) in the pooled faecal samples in the four subsequent herd cultures. The results of these 40 herds are presented in the table. Table: Numbers of positive pools in herds with culture of strategic pooled faecal samples at four subsequent herd investigations. Subsequent herd cultures firstsecondthirdfourth number of positive pools none per herd87776758 one per herd9577 two per herd2232 three per herd1100 four per herd1000 Besides the 13 herds that were excluded from the program after checking the registered laboratory records from the past, and the 40 herds that were positive at pooled faecal herd culture, another three herds had to be removed from the program because of closing down the herd or introducing animals of herds with a unknown paratuberculosis status. This means that from 113 participants after a two year program only 57 herds remained. Contamination of faecal cultures was responsible for a lower reliability of the negative culture results in three of these herds. Conclusions were: (1) Absence of clinical signs, as declared by the herdsman and confirmed by the practicing veterinarian, is no guarantee about the absence of Mptb infections in the herd. (2) Culture of pooled faecal samples was able to detect Mptb infections in a large proportion of herds unsuspected of paratuberculosis. (3) Repeated cultures of strategic pooled faecal samples in combination with closed herd management were needed to exclude Mptb infections in unsuspected dairy herds. The question how often cultures had to be repeated before herds could be declared free from paratuberculosis could not be answered from these results.

- Title** Johne's Disease and U.S. Cow-calf Operations.
- Author(s)** Dargatz DA, Wells SJ, Ott SL.
- Institution** Centers for Epidemiology and Animal Health, United States Department of Agriculture:Animal and Plant Health Inspection Service, Fort Collins, Colorado, USA.

Abstract The beef industry in the U.S. has prioritized Johne's disease as an issue for investigation. As part of a national study of health and management practices on U.S. cow-calf operations conducted by the National Animal Health Monitoring System (NAHMS) in 1997, data and serum samples were collected to provide information to the industry on producer awareness, prevalence, risk factors, and economic impact of infection. A stratified random sample of beef cow-calf operations was selected from 23 states to participate in the study. Questionnaire data were collected from 2713 producers (phase one) and 1190 producers (phase two). Data were weighted to generate population estimates for the inference population of each phase of the study; phase one - operations in the 23 states with 1 or more beef cows, phase 2 - operations in the 23 states with 5 or more beef cows. Study results show that overall the beef producer's awareness of Johne's disease was low with only 7.8% of producers having some knowledge of the disease beyond name recognition. Among those producers that at least recognised the name "Johne's disease" (30.1% of all producers), approximately 2 percent of producers reported a diagnosis of Johne's disease on their operation in the preceding 10 years based on any method of diagnosis. These study results indicate a need for broad industry educational efforts in order to successfully control Johne's disease in the U.S. beef cattle population.

Title Factors associated with Johnes disease on U.S. dairy operations.

Author(s) Wells SJ, Wagner BA, Dargatz DA.

Institution Centers for Epidemiology and Animal Health, U.S. Department of Agriculture: Animal and Plant Health Inspection Service: Veterinary Services, Fort Collins, Colorado, USA.

Abstract To help support activities of the National Johnes Working Group in the control of Johnes disease on U.S. dairy operations, one of the objectives of the National Animal Health Monitoring System (NAHMS) Dairy 96 Study, conducted by the U.S. Department of Agriculture: Animal and Plant Health Inspection Service: Veterinary Services, was to evaluate associations between specific herd management practices and Mycobacterium paratuberculosis herd infection status. Previous results from this study with over 1000 participant operations in 20 states showed at least 20% of U.S. dairy operations were infected with M. paratuberculosis and estimated the economic loss to heavily infected herds at over \$200 US per cow (infected and noninfected) per year. From multivariable analysis accounting for the study design, factors associated with herd M. paratuberculosis status included herd size, region, percent of milk cows born off the operation, use of multiple cow maternity housing, and use of multiple calf preweaned calf housing. Of particular value to Johnes disease educational efforts was the finding that familiarity or previous diagnosis with Johnes disease were not associated with use of preventive management practices.

Title Spreadsheet model for estimating the probability herds/flocks are free of paratuberculosis after successive serial tests.

Author(s) Collins MT.

Institution University of Wisconsin, Madison, WI 53706 USA

Abstract For herd certification programs tests for paratuberculosis typically are applied to all or part of the adult herd. Accuracy of herd classification is a function of the accuracy of the diagnostic tests employed, i.e., sensitivity and specificity. Test sensitivity and specificity, however, are estimated experimentally on the basis of correct classification of individual animals. A more appropriate measure of herd classification accuracy is herd sensitivity (Hse) and herd specificity (Hsp). These measures of test accuracy when tests are applied to herds incorporate the number of animals in the population tested and the prevalence of the infection in the population. Recent studies discuss the factors affecting Hse and Hsp. In most herd certification programs, tests are applied to herds or flocks repeatedly, usually on an annual basis, to gain confidence in the infection-free status of the animal population (serial testing). Decisions regarding which tests to use, how many times to test the herd/flock, and how many animals to test each year are difficult to make and there are no statistical models on which to base decisions. Program design is always a compromise between the degree of confidence in the infection-free status of herds/flocks and the cost of the testing required. To facilitate design of regional or national herd certification programs for paratuberculosis a spreadsheet model was developed that incorporates test sensitivity, test specificity, number of animals tested in the population, testing costs for the herd, estimated herd/flock prevalence of paratuberculosis, and estimated mean within herd prevalence of paratuberculosis for infected herds. From these parameters, the probability that herds/flocks are not infected with *M. paratuberculosis* and cost to the herd owner was calculated after each successive annual test. The gain in certainty (post-test probability not infected minus pre-test probability not infected) is calculated in the spreadsheet as is the cost per unit gain in certainty. Lastly, for national program design purposes, the model calculates the cost to the state or country for implementation of the program used based on mean herd/flock size and numbers of herds/flocks in the state of country. The concepts behind the model will be described and the influences of altered assumptions of test accuracy or test selection will be demonstrated.

Title Sample survey for estimating the herd and individual animal seroprevalence for bovine paratuberculosis in Belgium.

Author(s) Boelaert F, Walravens K, Vermeersch JP, Raskin A, Dufey J, Godfroid J.

Institution Veterinary and Agrochemical Research Centre, Groeselenberg 99, 1180 Ukkel, Belgium.

Abstract From December 1997 to March 1998, a sample survey was conducted in Belgium to estimate the national bovine paratuberculosis (PTB) herd and animal seroprevalence. This survey aimed to provide an unbiased estimate of the PTB seroprevalence, as the sampling units were randomly selected using the co-ordinates for the cattle herds registered in SANITEL-Cattle, the central computerised database for the identification and registration of cattle in Belgium. A stratified sample design was followed. The total number of herds to be sampled was set at 1 % of the total number of Belgian cattle herds, and the number of herds to be sampled per province was determined by proportional allocation. In the selected cattle herds, all adult cattle of 2 years or older were blood sampled. A herd was defined to be PTB-seropositive if at least one PTB-seropositive adult bovine was present. The serum samples were tested for antibodies to PTB, using a commercially available Absorbed ELISA test-kit (HerdChek, IDEXX, France). Seventy seven of the 442 herds had at least 1 PTB-seropositive adult cow. Consequently, the PTB herd seroprevalence was 17.42 %. The individual animal seroprevalence was 1.18 %; 143 of the 12,077 adult cattle were seropositive to PTB. Detailed analysis of the seroprevalence for different herd and management characteristics are in progress.

Title An interactive model of Johne's Disease risk and prevalence for use in educational programs.

Author(s) Elrod C, Rossiter C, Collins MT, Pollak JP.

Institution Cornell University, Ithaca NY and University of Wisconsin, Madison WI, USA.

Abstract Dairy producers and other dairy industry professionals are generally unaware of the progression or implications of Johne's Disease within a herd. Likewise, they are unfamiliar with the efficacy

of various intervention practices. The simulation which we have developed couples a detailed risk assessment tool with a herd prevalence model. The resulting simulation allows us to assess or describe a farm situation in six management areas related to Johne's Disease risk: the maternity pen, pre-weaned calves, calves to six months old, breeding age heifers, bred heifers and cows. Next, by describing herd demographics, cattle importation practices and known Johne's Disease prevalence, the simulation produces a projection of prevalence over the next twenty years.; The learner can then select to adopt a test and control plan or to implement intervention practices. By choosing the management area(s) on which they wish to focus and the level of intensity of their interventions, the simulation allows users to project the course of Johne's Disease prevalence over the ensuing twenty years. Through this exercise, learners come to appreciate the relative effectiveness of various management strategies, the time course necessary for Johne's Disease control and the necessity of adopting a systematic, long range plan for control. This simulation will be one component of a larger computer aided instructional program on Johne's Disease.

Title Paratuberculosis management risk factors on dairy farms in The Netherlands.

Author(s) Muskens JAM, Jongeneel D, Verhoeff K.

Institution Animal Health Service, PO Box 4, 5280 AA Boxtel, The Netherlands.

Abstract Nature of paratuberculosis infection and characteristics of available tests block a simple test and cull approach in a paratuberculosis control program. Special attention is necessary to prevent spread of *Mycobacterium paratuberculosis* in and between herds especially to young stock. In this respect the daily farm management is of great importance. A survey of relevant management factors was carried out in 379 randomly selected herds out of all 28,918 herds with 20 heads of dairy cattle or more in The Netherlands. An Animal Health Service staff member visited the selected herds with a questionnaire during the 1998 summer season. In 62 per cent of cases birth takes place in a separate calving room. Special hygienic precautions (mainly washing and disinfection of the posterior part of the cow) are taken in 19.2 per cent of the calvings. Replacement calves are reared in 97.3 per cent of the herds. After birth 53.8 per cent of the farmers immediately separate the calves from their mothers. Special hygienic precautions in calf rearing (clean boots at the entrance of the calf house) are practised in 12.4 per cent of the herds. A calf is supplied with colostrum from several dams by 15.5 per cent of the farmers, while 76.3 per cent of the farmers supply fresh milk from different cows (for instance milk containing antibiotics) at least occasionally. 56.9 per cent of calves are housed during the first 6 months of life and 25.8 per cent during the first 12 months. Of the calves at pasture during the first 6 months, 12.4 per cent graze pastures previously grazed by dairy cows. Clinical paratuberculosis cases have been observed in 14.1 per cent of the herds during the past 3 years. It is concluded that presently farm management of many farmers is inadequate to prevent spread of *Mycobacterium paratuberculosis* to young stock. Especially the probability of getting infected shortly after parturition by contaminated faeces, colostrum or milk is alarming.

Title Control and eradication of bovine Johne's disease.

Author(s) Kluver PF, Condrón RJ, Hope AF, Carajias M, Ridge SR¹, Tulk PA, Waldron BR.

Institution Victorian Institute of Animal Science Attwood, ¹ Department of Natural Resources and Environment Leongatha Victoria Australia.

Abstract A Johne's disease control program based on herd testing with the Johne's absorbed EIA (PARACHEK®) and faecal culture was implemented in 25 Victorian dairy herds in 1990. Recognised management strategies to control disease were adopted and test positive animals were removed annually prior to the calving period. After three years the average herd prevalence was reduced. Annual serological and faecal culture testing was continued in eleven herds. Progress in disease control was influenced by the effectiveness of calf management and hygiene, the introduction of infected cattle to the herd, the effectiveness of diagnostic tests to detect infected animals and the timeliness of culling reactors. A review of progress in these herds over the past 8 years will be presented in the paper.

Title Herd Testing to Control Bovine Johne's Disease

Author(s) Galvin JW, Jubb TF.

Institution Department of Natural Resources and Environment Box 2500 Bendigo Victoria 3554

Abstract There are about 1,800 cattle herds in Victoria in which Johne's disease (JD) has been diagnosed in the previous 5 years, the majority of which are dairy herds. The costs associated with JD include production losses and trade restrictions. Stud and elite breeding herds are the most financially affected. Traditional control methods rely on management practices to reduce the opportunity for calfhood infection. These include avoiding contact with adult cattle, pastures grazed by adult cattle and effluent from dairy sheds. The absorbed ELISA blood test is able to detect up to 50% of infected cattle over 2 years of age and can be used in conjunction with management practices to improve JD control, however it requires a commitment over many years by herd owners. In 1996, the Victorian cattle industries and Government established a JD Test and Control Program under which participating farmers are provided with an annual ELISA test of their adult herd and advice on disease control that is tailored to their farm. The program is delivered through private veterinarians under contract. There are over 500 herds enrolled in the program and about one third of these have had 3 or more whole herd tests. The paper provides a review of the program to date. It describes the trends of ELISA reactor rates and clinical cases, and some factors affecting JD control in participating herds.

Title Paratuberculosis in Iceland; Epidemiology and control measures, past and present.

Author(s) Friðriksdóttir V, Gunnarsson E, Sigurðarson S, Gudmundsdóttir KB.

Institution

Abstract Paratuberculosis as well as Maedi/visna and Jaagsiekte came to Iceland in 1933 when 20 sheep of the Karakul breed were imported from Halle, Germany. At least 5 of these sheep were healthy carriers of paratuberculosis. In a matter of 16 years Paratuberculosis together with the other Karakul diseases (Maedi/visna and Jaagsiekte) almost ruined sheep farming, the main agricultural industry in Iceland. The first case of paratuberculosis in sheep was confirmed in 1938 and in cattle in 1944 on farms where the disease had been prevalent in sheep for years. The virulence in cattle appeared to be considerably lower than in sheep. Extensive measures were used to control the spread of paratuberculosis in sheep. Hundreds of kilometres of fences were put up and used together with natural geographic hindering to restrict the movement of sheep from infected areas. Serological and other immunological tests were furthermore used to detect and dispose of infected individuals. These measures were inadequate and the disease could not be eradicated. Extensive culling of sheep in endemic areas and restocking with healthy ones eradicated Maedi/Visna and Jaagsiekte but not paratuberculosis, whereas vaccination reduced mortality by 94%. Vaccination of sheep has been compulsory in Iceland since 1966 in endemic areas and as a result losses have been reduced considerably. Today serology is used on cattle to detect and control infection, and on sheep to control vaccination and screen for infection in non-endemic areas. The CF (complement fixation) test for paratuberculosis has been used until now, but recently we have started comparing the CF test with the CSL absorbed ELISA test. The parallel testing of sera with the CF test and the absorbed ELISA test is still going on and preliminary results will be presented.

Title Bovine paratuberculosis and tuberculosis in a field study.

Author(s) Bernardelli A, Torres P.

Institution National Service for Food and Agriculture Health and Quality, SENASA, Requena J. Private Veterinary - Argentina.

Abstract In a herd with bovines by *Mycobacterium bovis* and *Mycobacterium avium* subsp. paratuberculosis of clinical diagnostic of diarrhoea, 137 Brangus bovines from a Buenos Aires, province farm, where tuberculinized, using the tuberculin technique of Delayed-type

hypersensitivity (D.H.T.) with Bovine P.P.D., 1 mg/ml, (32.500 U.I) and Avian P.P.D., 0.5 mg/ml, (25.000 U.I.) a prevalence of 45.9% for Paratuberculosis and 27.0% for Tuberculosis were detected. The interpretation of the Comparative Test is based on the size of the response to the bovine tuberculin compared to the avian tuberculin. At the herd level, the scattergram is used for the interpretation of the results of Cervical Comparative, classifying the animals in different groups. The AGID method indicated 267 negative sera and 35 positive sera for paratuberculosis. The bacteriology isolating from four bovines in Herrold medium with mycobactin were obtained from the mesenteric lymph node, ileum caecum valve, caecum, ileum, rectum and faeces. The analysis of sera using the ELISA, ELISA absorption with *Mycobacterium phlei*, gamma interferon and tuberculin tests, gave results that were coincident and discordant with relation to the positivity and negativity of the samples for these two diseases. The appearance of *Mycobacterium bovis* and *Mycobacterium avium* subsp. paratuberculosis in a herd, a situation that is frequent in some regions, represents a problem for a diagnostic that requires great effort towards the management and control of these diseases.

- Title** Control of paratuberculosis in five cattle farms with 1700 cows from 1990-1998 by serologic tests and faecal culture.
- Author(s)** Pavlík I, Matlova L, Vesely T, Bartl J, Valent L¹, Miskovic P², Hirko M².
- Institution** Veterinary Research Institute, Brno, Czech Republic. ¹ Regional Veterinary Administration, Dunajska Streda, Slovak Republic. ² Field veterinarian of the relevant farm.
- Abstract** In 1989, clinical paratuberculosis was detected in the Slovak Republic from two cows on one farm I (out of five farms from one herd) surveyed. Gradually the disease spread to all five farms, designated as I-V containing 1700 cows, 1100 calves and 1300 heifers. The origin of the infection was most probably the importation of 60 heifers of the Holstein breed from Denmark to farm V in the late 70's. In the mid of 80's, due to the common housing of heifers, the remaining four clean herds were also infected with paratuberculosis. In 1990, an eradication and control program against paratuberculosis was commenced. In the first phase (up to 1992), all animals > 18 month old, were tested by three serologic examinations using two different methods. Animals with repeatedly positive serologic results, including the clinically suspected ones were culled from the herd. In the second phase (from 1992) faecal examinations were performed twice a year in addition to serology. Altogether, 15,951 faecal samples were cultured, 30,346 examined by AGID, 20,486 by ELISA and 9,860 by CFT. In the early of 1989-1990, the prevalence of clinical paratuberculosis in farm I-V varied (I-9.4%, II-6.3%, III-0%, IV-0.6% and V-0%). According to the extent of the infection, we divided 366 infected animals into three groups: a) clinically healthy -51.4%, b) clinically healthy with progressive infection -23.5%, c) clinically sick with severe infection - 25.1%. The percentage of culled animals (from 1990-1992) in each category was: a) 22.5%, b) 35.0%, c) 42.5%. Faecal culture, however, revealed that the number of infected animals in group a) increased from 22.5% to 60.0%. Thus, faecal surveillance enabled us to cull individuals which were in the preclinical stage of the disease. Accordingly, the incidence of the disease in 1997 in farm I-IV was < 1.5%. However, in farm V the incidence was still high which could have been due to the common housing of calves with cows between 1993-1996. Our research was partially supported by the Ministry of Agriculture of the Czech Republic (grant no. EP0960006087) and Czech Grant Agency (grants No. 514/95/1594 and 524/97/0948).

- Title** Poster Presentation An epidemiological study of paratuberculosis in wild rabbits in Scotland.
- Author(s)** Stevenson K¹, Greig A², Henderson D², Pérez V^{1,3}, Hughes V¹, Pavlík I⁴, Hines II ME⁵, McKendrick I⁶, Sharp JM¹.
- Institution** ¹ Moredun Research Institute, International Research Centre, Pentland Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ, Scotland, UK. ² SAC Veterinary Science Division, Cleve Gardens, Oakbank Road, Perth PH1 1HF Scotland, UK. ³ Present address, Histologia y Anatomia Patologica, Facultad de Veterinaria, Universidad de

Leon, Spain. ⁴ Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic. ⁵ Veterinary Diagnostic and Investigational Laboratory, University of Georgia, 43 Brighton Road, Tifton, GA 31793 USA. ⁶ Biomathematics and Statistics Scotland, The King's Buildings, Edinburgh EH9 3JZ Scotland, UK.

Abstract A survey of 22 farms confirmed the presence of paratuberculosis in wild rabbits in Scotland. Regional differences were apparent in the prevalence of the disease in rabbits identifying a significantly higher incidence in the Tayside region. Statistical analysis showed a significant relationship between a previous history or current problem of paratuberculosis in cattle and the presence of paratuberculosis in rabbits on the farms. Molecular genetic typing techniques could not discriminate between selected rabbit and cattle isolates from the same or different farms suggesting that the same strain may infect and cause disease in both species and that inter-species transmission may occur. The possibility of inter-species transmission and the involvement of wildlife in the epidemiology of paratuberculosis have important implications for the control of the disease.

Title Johne's disease in Austrian cattle: a seroprevalence survey.

Author(s) Gasteiner J, Wenzl H, Fuchs K, Jark U, Baumgartner W IInd.

Institution Medical Clinic for Ruminants and Swine, University of Veterinary Medicine, Vienna, Austria; Joanneum Research Institute GesmbH., Graz, Austria; Institute of Microbiology and Infectious Animal Diseases, University of Veterinary Medicine, Hanover, Germany.

Abstract From 1995 to 1997 the prevalence of serum antibodies against *Mycobacterium avium* subspecies paratuberculosis (*M. av. ssp. pt.*)- the causal agent of paratuberculosis (Johne's Disease) - was examined in 11,028 Austria cattle. Samples of the 4 oldest cattle of 2,757 farms were collected according to a specific sampling schedule for this epidemiological study. District, age and breed of animals were included in this study. For antibody screening against *M. avium* subspecies paratuberculosis a modified, commercially available ELISA (ALLIED Monitors, Fayette, USA) was employed. 2,253 samples that were found to be positive or questionable were subjected to further testing with a more specific ELISA (Institute of Microbiology and Infectious Animal Diseases). Results of this study were used for statistical analysis. Average prevalence of antibodies to *M. avium* subspecies paratuberculosis was 1.99 % in Austria. Highest prevalence was seen in 6 year old cattle (2.84 %) and Holstein Frisian cattle (3.51 %). Seropositive animals were found in 6.96 % of farms tested, and prevalence was highest in Vorarlberg, followed by Salzburg, the Tyrol, Styria and Carinthia. This study is unique in Europe in the use of an adequate random sampling plan for an investigation of this magnitude.

Title Prevalence and Regional Distribution of Bovine Paratuberculosis in the Netherlands

Author(s) Muskens J, Barkema HW, Russchen E.

Institution Animal Health Service, P.O. Box 4, 5280 AA, Boxtel, The Netherlands

Abstract In the Netherlands an eradication program of *Mycobacterium paratuberculosis* (Mptb) is considered. The design of this program depends highly on the herd and cow prevalence of Mptb. To estimate this prevalence, out of the 28918 dairy herds with > 20 cattle older than 2 years, a sample of 460 herds was selected. Herds were randomly selected in the four regions (North, East, West, and South) with an equal proportion. In total 378 farmers, 91-97 per region, decided to participate. Serum samples of all cows older than 3 years of age were collected in the period July - October 1998. The farmers were asked if the cattle was vaccinated against paratuberculosis. Serum samples were tested using the Ubitech (Idexx) absorbed ELISA. Serum samples with a Sample to Positive ratio > 0.3 were regarded as positive. Out of a total of 15822 cows, 520 animals were tested positive. Taking into account the regional distribution of the number of herds as well as the herd size, it was calculated that 3.3% of the cattle in the Netherlands was serologically positive. The percentage positive cows differed between regions. In the Northern region, for example, 5.0% of the cows were tested as positive, whereas in het

Western region only 2.2% were tested positive. The percentage herds with at least one serologically positive cow was calculated to be 55%. In the Northern region the percentage herds with at least one positive cow was the highest (69%) and in the Western region the lowest (44%). Of the serologically positive herds (n = 206) 96 herds had only one positive cow whereas 49 herds had 2 positive cows. Eleven of the herds tested, were vaccinated against paratuberculosis and had a total of 95 positive cows (range 1-28 animals per herd). Eight of these herds belonged to the Northern region.

Title Paratuberculosis Infection of Sheep on Farms with Paratuberculosis Infected Cattle in the Netherlands

Author(s) Muskens J¹, Bakker D², de Boer J¹.

Institution ¹ Animal Health Service, P.O. Box 4, 5280 AA, Boxtel, The Netherlands ² ID-DLO, Lelystad, The Netherlands

Abstract Grazing of cattle and sheep on the same pastures is not uncommon in the Netherlands. Until now these sheep play no role in the control measures proposed for the prevention of paratuberculosis in cattle. A thorough investigation of sheep on farms with a known history of paratuberculosis in cattle was instigated to assess the validity of this approach. Early 1998, 18 farms with both sheep and cattle were selected for further study. Selection was based on a positive paratuberculosis diagnosis in one or more cows during the years 1996-1998. Cattle and sheep on these farms were grazing on the same pastures or the sheep pastures were fertilised using manure of the cattle. All sheep more than one year of age (n = 919) were serologically tested using an absorbed ELISA (Ubitech). The number of sheep per flock varied from 15 to 133. Serum samples with an S/P (Sample to Positive) ratio > 0.3 were regarded as positive. Five flocks had 1 serologically positive sheep, all the other samples tested negative. The 5 positive sheep and 45 others, based on the condition of the animal, most suspected sheep, were purchased from 15 farmers. The number of purchased sheep per flock varied from 2-6. These 50 sheep were necropsied in the period June-September 1998. Extensive examination of jejunum, ileum, ileocaecal valve, ileocaecal, mesenteric and retropharyngeal lymph nodes and intestinal contents, was performed by means of culture, histology and Polymerase Chain Reaction.

Title Is paratuberculosis in goats a source of infection to cattle? Some preliminary results from a national surveillance program in Norway.

Author(s) Djønne B¹, Holstad G¹, Kolbjørnsen ¹, Nyberg O², Schönheit J¹, Tharaldsen J¹, degaard .

Institution National Veterinary Institute, ¹ Oslo/² Bergen, Norway

Abstract A national surveillance program on paratuberculosis in cattle was started with a preliminary serological survey on imported herds in Norway in 1997. So far, only herds with recently imported cattle, herds with both cattle and goats and herds with a certain number of animals above 7 years of age have been included. The survey was based on the enzyme-linked immunosorbent assay (ELISA). Several of the seropositive animals were slaughtered and pathological and bacteriological examinations of the intestine were carried out. Animals from seropositive herds were in addition examined by faecal culture. A few seropositive animals were detected among recently imported cattle. The positive serological results were later confirmed by pathological and/or bacteriological findings in 7 cattle from 4 herds. These animals could be traced back to two different imports in 1992 and 1994, respectively. In Norway, bovine paratuberculosis has not been diagnosed between 1979 and 1992. In goats, however, the disease has been a consistent problem for several years, the disease has been controlled by a vaccination program. The mycobacterial isolates from goats have been considered non-pathogenic for cattle (Saxegaard 1990). However, several seropositive cattle were demonstrated in herds with no history of imported animals. These animals had no clinical signs of paratuberculosis, but most of them came from herds with close contact between cattle and goats. Some of the seropositive animals were examined by pathology and bacteriology. The results of these investigations will be presented.

Title Mycobacterium paratuberculosis infection in a water buffalo (*Bubalus bubalis*) from Central Italy.

Author(s) Lillini E, Gamberale F, Di Guardo G.

Institution Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Rome, Italy

Abstract Paratuberculosis, a naturally occurring disease of domestic and wild ruminants, has been reported in cattle, sheep and goats in Italy. Water buffalo (*Bubalus bubalis*) is another economically important species in our Country, especially in Latium (Central Italy) and Campania (Southern Italy). Following obtainment of a financial aid on a research project aimed to animal productions' improvement, which has been issued by the European Union (5b Project), 15 water- buffalo herds from three different provinces of Latium region (Frosinone, Latina, Rome) were investigated between 1997 and 1998. More in detail, a total number of 1,321 animals were submitted to serological tests for *M.paratuberculosis* antibody detection (AGID and ELISA). Three adult subjects, all belonging to an identical farm, turned out to be positive. Only one of these, a 5- years-old female, showed clinical signs related to the disease, namely progressive emaciation, weakness, chronic intermittent diarrhoea, reduced milk production, and infertility. The disease started in November 1997 and a second blood sampling was carried out 7 months later on all three concerned subjects, the remaining two of which showed, however, no evidence of clinical disease. At the same time, faecal samples were collected from the three animals. Detection of several aggregates of acid-fast bacilli with mycobacterial morphology was achieved only in the clinically affected subject, which is still alive. On the other hand, all three investigated buffaloes remained seropositive. Microbiological investigations for *M. paratuberculosis* on faecal samples have so far yielded negative results. To our knowledge, this is the first case of *M. paratuberculosis* infection in water buffaloes from Italy.

Title IS900 Restriction Fragment Length Polymorphism of Australian isolates of *M. paratuberculosis*

Author(s) Cousins D¹, Williams S¹, Hope A², Eamens G³.

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Abstract Although epidemiological evidence supports the fact that sheep and cattle are infected with different strains of *M. paratuberculosis* in Australia, this had not been confirmed. This work was designed to examine whether sheep strains in Australia are the same as are found in New Zealand, and whether the polymorphism's detected in Australian isolates of *M. paratuberculosis* would be useful in epidemiological investigations of Johne's disease in Australia. Seventy three isolates of *M. paratuberculosis* from 66 animals were examined for genetic variation using the insertion sequence IS900. The samples originated from 5 animal species in 5 Australian States and the Northern Territory. Four reference strains and an isolate from a Crohn's disease patient were also examined. The 3 restriction enzymes most commonly used from IS900 RFLP were used, and 4 additional restriction enzymes were assessed. *Bst* EII and *Bam* HI proved to be the most useful for detecting polymorphisms in Australian strains of *M. paratuberculosis*. In one case, isolates with a single band difference were found from different sites in the same animal. Most cattle and some other species are infected with cattle (C) strains and sheep were predominantly infected with sheep (S) strains. Thus, while the C strain infected other animal species such as alpaca, goats, a rhinoceros and sheep, there was no evidence in this study of the S strain infecting cattle. Because of the small number of C strains that had unique IS900 RFLP patterns, it is unlikely that IS900-RFLP would be useful in epidemiological investigations of Johne's disease transmission amongst cattle. Since no distinctions could be made among ovine isolates, this fingerprinting technique is unlikely to be of use in the investigation of ovine Johne's disease epidemiology other than to clarify whether infection on newly diagnosed properties was due to members of the C or S groupings.

Title Usefulness of pulse-field gel electrophoresis in the epidemiology of human and animal isolates of *Mycobacterium paratuberculosis*.

Author(s) Ackerman A, Lambrecht RS.

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Abstract DNA fingerprints were generated using PFGE following extraction of mycobacterial DNA and digestion with the restriction endonucleases Xba-1 and Vsp-1 for many of the known human paratuberculosis isolates (Holland, Holland-1, Holland-2, Linda, Dominic and Ben) and the type strain ATCC 19698. Preliminary results indicate that three isolates (Linda, Dominic, Holland-1 and the type strain) demonstrated genetically identical PFGE fingerprint patterns when cut with either Xba-1 or Vsp-1. Fewer bands were observed with Vsp-1 compared with Xba-1, but both were useful in determining whether strains were similar or different. All strains could be placed into 4 distinct groups based on their PFGE fingerprint patterns. In addition, we have used PFGE to examine a number of *M. paratuberculosis* isolates from different animals. An isolate from a Wisconsin farm demonstrated the same identical PFGE pattern as an isolate obtained from a Florida Zoo using the enzyme Xba-1, whereas, there was a single band difference between these same isolates with Vsp-1. We conclude that PFGE is a useful tool in differentiating genotypic relationships among *M. paratuberculosis* and is helpful in providing evidence for strains being epidemiological linked.

Title The New South Wales Johne's disease information management system.

Author(s) Sergeant ESG.

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Abstract Since the early 1990's, there has been an increasing number of cases of Johne's disease diagnosed in both sheep and cattle in NSW. This has resulted in extensive changes in attitude to Johne's disease by industry, and an increased regulatory policy implemented by NSW Agriculture. Current policy for both sheep and cattle includes identification and investigation of high risk flocks, active tracing of movements into and out of infected herds and flocks and movement restrictions on infected herds and flocks. To manage the large amount of data generated by these activities an information management system for Johne's disease was developed. This is a relational database system, consisting of seven individual databases, developed in Epi Info v6, a DOS program for relational database management and epidemiological analysis of data. Data is recorded for individual properties, including current property status, history of status changes, investigations and testing undertaken, quarantines and notices issued, and tracings (stock movements) identified. Data is entered and managed on PCs at the local level by district veterinarians, with regular (monthly, or by request) updates of data at regional and state level. All records are uniquely identified to district and property level. The State database is an amalgamation of all data files from all districts for the State. The system is menu driven, and produces a range of pre-programmed reports at the State level for management of the program, monitoring of progress and epidemiological analysis, as well as reports at the local level to assist local management of the disease. Customised reports can be developed and saved for re-use using Epi Info reporting tools. Data from the system is being used to manage the OJD control program in NSW, and to model spread of the disease and predict its extent in NSW.

Title Evaluation of the long term of the immune response in cattle after vaccination against paratuberculosis in two Dutch dairy herds

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Abstract In the Netherlands, vaccination against paratuberculosis in cattle is performed on a limited scale. Because of its interference with the diagnostics for bovine tuberculosis, vaccination is restricted to herds with a high prevalence of paratuberculosis and is meant to aid in the economical survival of the farm. At present plans are being made to begin with a nationwide certification program for paratuberculosis. Since serological methods are likely to part of the first steps in any conceivable certification program, herds that have been vaccinated in the past are likely to encounter problems when entering such a program. The aim of this study was to evaluate the immune response resulting from vaccination using a heat killed vaccine. Two farms, one with 102 animals and one with 208 animals were selected. On both the vaccination program started more than 9 years ago; the larger farm is still vaccinating, whereas the smaller one stopped vaccinating two years ago. The humoral immune response was evaluated in all animals using the CFT test and the absorbed ELISA, the T-cell response using the gamma-interferon assay with bovine and avian PPD and Johnin as stimulants. Furthermore, faecal culture was performed on all animals to assess the influence of possible lingering paratuberculosis infection in the herds on the immune status of the animals. The data obtained show a prolonged effect of the vaccination on both the cellular- and the humoral immune response, in particular to the avian- and paratuberculosis antigens. Implications of these results for the certification of these herds will be discussed.

Title Vaccination of Cattle against Paratuberculosis with an Inactivated Vaccine. A controlled field study in an infected herd.

Author(s) López Cruz A¹, Perales Flores A², Sánchez-Prieto Borja M², Franco Cayón FJ³, Puentes Colorado E¹.

Institution CZ Veterinaria, S.L., Porrio. ¹ Laboratorio Nacional de Sanidad Animal, Santa Fe, Granada. ² Servicio Territorial de Agricultura y Ganadería, Junta de Castilla-León. ³ Spain.

Abstract The efficacy of an inactivated vaccine against paratuberculosis, GUDAIR(r), was evaluated in a naturally infected herd of cattle. All the animals from the herd were vaccinated and a representative group of 100 animals was used for the study. Serum and blood samples were taken on the day of vaccination and at 21, 60, 120, 180, 240 and 300 to evaluate the humoral and cellular immune response. The intradermal tuberculin test was performed at day 0, 60, 180, 240 and 300 post vaccination. Adverse effects caused by the vaccine were not observed except for persistent nodules at the vaccination site. The vaccine produces a strong and lasting cellular and humoral immune response. Vaccinated animals reacted to both bovine and avian PPD tuberculins, but the avian reaction was usually stronger and persisted longer than the mammalian one. The vaccine highly reduced the losses due to the disease. Three months after vaccination no clinical signs were observed and a high increase in milk production was recorded. During the last four years all new replacement animals have been vaccinated and no animals with clinical paratuberculosis have been notified.

Title Differences in the immune responses in lambs and kids vaccinated against paratuberculosis, according to the age of vaccination.

Author(s) Corpa JM, Pérez V, García Marín JF.

Institution Departamento de Patología Animal: Medicina Animal (Anatomía Patológica). Facultad de Veterinaria. Universidad de León. Campus de Vegazana, s/n. 24071 León (Spain).

Abstract Vaccination has been widely used for the control of paratuberculosis. Traditionally, immunisation has been practiced during the first weeks of life under the hypothesis that infection takes place soon after birth. However, it has been demonstrated that vaccination does not prevent infection but modifies the host responses towards a limitation in the progression of lesions. Additionally, there are some evidences in tuberculosis suggesting that vaccination gives better results when it is carried out in animals around 5-6 months old. In this study, the cellular and humoral immune responses have been evaluated by means of gamma-IFN and ELISA tests respectively. Cell populations in circulating blood were studied by FACS analysis. Three flocks

of sheep and three of goats have been used. In every flock, two groups of animals were made according to the age of vaccination (10-15 days and 4-5 months). Every group was composed of 15 animals each, in which 10 were vaccinated with a killed vaccine (Gudair(r)) and 5 kept as control. Both groups of animals showed a similar kinetics in the production of antibodies and gamma-IFN; however these responses appeared before, reached higher values and lasted for longer periods of time in animals vaccinated at 4-5 months than in younger animals.

Title Evaluation of two vaccines (killed and attenuated) against small ruminant paratuberculosis.

Author(s) García Marín JF, Tellechea J, Gutiérrez M, Corpa JM, Pérez V.

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Abstract Vaccination has been widely used as a procedure for the control of paratuberculosis. Both killed and attenuated vaccines have been employed in several experiments with generally good results. In this work, the cellular (measured by intradermal skin test and gamma-Interferon assay) and humoral (estimated by means of AGID and ELISA tests) responses have been evaluated in lambs and kids vaccinated with an attenuated (Neoparasec(r)) or a killed vaccine (Gudair(r)). These animals constituted the replacement of two flocks of sheep and one of goats with annual losses due to paratuberculosis %gt; 6%. Immune responses followed a similar pattern in both vaccines. Very high cellular and humoral reactions appeared at 15-30 days post vaccination (d.p.v.) which were increasing until 100 d.p.v. From then, a slow decrease was observed until the 300 d.p.v. when the last sampling was performed. A clinical and pathological follow-up was carried out in these flocks for the following two years and no new cases of paratuberculosis were diagnosed. No significant differences were detected either in the immune responses or in the efficiency of the two types of vaccines employed.

Title First evidence of paratuberculosis in farmed red deer (*Cervus elaphus*) in Belgium.

Author(s) Godfroid J, Boelært F, Desmecht M, Walravens K.

Institution Veterinary and Agrochemical Research Centre, Groeselenberg 99, 1180 Ukkel, Belgium.

Abstract In order to assess the prevalence of paratuberculosis in a deer farm, direct and indirect tests were performed on 24 young animals (yearlings and 2 years old animals). There was an history of diarrhoea last year in animals imported from the United Kingdom. These animals were slaughtered without any definitive diagnosis but faecal samples were taken for further bacteriological examination. The following tests were performed: serology (*M. paratuberculosis* Ac-ELISA, Idexx), comparative cervical skin test using *M. bovis* PPD and *M. avium* PPD, using *M. bovis* PPD, *M. avium* PPD as antigens as well as PMA - Ionomycin and *M. phlei* PPD respectively as positive and negative controls. Microscopic examination after a Ziehl-Neelsen staining was also done. Four positive serological results, 3 positive skin tests and 3 positive lymphoproliferation tests were observed in animals older than 2 years. No positive serological results were observed in the yearling group whereas some sensitisation were observed in the skin test as well as in the lymphoproliferation test for the same group of animals. The degree of concordance between these indirect tests was poor. This can be explained partly because of the pathogenesis of the disease but also because of the absence of data according to the intrinsic values of these tests in wild farmed species. A Ziehl - Neelsen staining yielded a positive result on the animal already slaughtered. Three seropositive animals were slaughtered and histopathology was performed on mesenteric lymphnodes and on the ileon. Although no Ziehl-Neelsen staining yielded positive results, a catarrhal focal necrotic enteritis associated with a granulomatous lymphadenitis compatible with paratuberculosis as been evidenced. The bacteriology on all samples is pending. All together our results suggest that paratuberculosis is present in this deer farm, although the true intra-herd prevalence has still to be assessed.

Title Control of paratuberculosis in two goat flocks.

Author(s) Vélez-Hernández M, Domínguez-Punaro M, Chávez-Gris G, Suárez-Güemes F.

Institution Facultad de Medicina Veterinaria y Zootecnia. Universidad Nacional Autónoma de México. Circuito Exterior s/no. Ciudad Universitaria 04510, Coyoacán, México City.

Abstract In order to stop the spread of paratuberculosis in 2 goat flocks (I,II) a control program was established and followed for 2 years. Prior to establishment of the "Test and Cull" control program both flocks demonstrated similar prevalences of paratuberculosis: 6.39% and 4.89% respectively. Subsequently, flock II demonstrated a significant decrease ($\alpha=0.01$) in its prevalence of paratuberculosis. In flock I the paratuberculosis prevalence increased significantly ($\alpha=0.01$). Because of the increasing prevalence in flock I, a vaccination program was adopted.

Title Shedding of organisms and sub-clinical effects on production in pre-clinical Merino sheep affected with ovine paratuberculosis.

Author(s) Abbott KA, Chaitaweesub P.

Institution Department of Veterinary Clinical Sciences, University of Sydney, Camden, Australia.

Abstract One hundred 12 month old Merino wether sheep from a flock with longstanding ovine paratuberculosis were studied until they were 22 months old. Serum samples and faecal samples were collected every 6 weeks for 10 months and liveweights were recorded. Fleece weight and average fibre diameter were recorded at 19 months of age. Serum samples were tested with an ELISA. At the beginning of the experiment, no sheep had ELISA OD ratios greater than 2.4 but by 22 months 18 sheep had ratios greater than 2.4 or had had on at least one occasion. These 18 sheep were killed and necropsied and paratuberculosis status was determined by histopathology. 6 sheep were found to be affected. Retrospective culture of the faeces from these sheep was used to determine when faecal shedding commenced. There was no effect of sub-clinical infection on wool production but an effect on liveweight was evident from about the time when serology was positive.

Title Surveillance for Ovine Johne's Disease in New South Wales - Analysis of Results of Laboratory Submissions.

Author(s) Links IJ*, Moloney B, Reddacliff L, Sergeant E.

Institution NSW Agriculture, Wagga Wagga*, Australia

Abstract Ovine Johne's Disease was first diagnosed in NSW in 1980. An intensive surveillance program was carried out from April to July 1998 to better define the distribution and prevalence of the disease. More than 800 investigations were made on over 650 flocks from throughout NSW. The gel test was performed on more than 100,000 blood samples with reactors necropsied and intestinal sections submitted for histopathological examination. Blood samples were derived from two main flock categories - those identified as "at risk" because they had purchased sheep from or had other contact with known infected properties or those with no known risk factors that wished to demonstrate their freedom from the disease (eg Market Assurance Program testing). Analysis of the results of serological and histopathological testing will be reported. They provide a useful guide to the testing strategies most suited to a surveillance program of this type.

Title Ovine Johne's disease: the risk associated with sheep imported into Western Australia.

Author(s) Higgs ARB, Hawkins CD.

Institution Agriculture Western Australia, Albany, Australia.

Abstract Johne's disease is not known to occur in Western Australia. For the sheep industry, the most significant risk comes from the importation of sheep from interstate. The risk of Johne's disease not being detected in sheep imported from New South Wales was assessed quantitatively by using a stochastic simulation model. Results from the computer model were used to support decisions about controls on the importation of sheep. The process of importing sheep was broken down into steps and numbers or probabilities assigned to each. Controls on the movement of sheep included surveillance tests in source flocks and serological tests on sheep in consignments before and after transportation to Western Australia. The model calculated the risk of occurrence of Johne's disease in Western Australia and the success of the agar gel immunodiffusion test in identifying consignments with infected sheep. Negative surveillance tests in source flocks reduced the risk to about one twentieth of that when no surveillance tests were required. On average, Johne's disease was predicted to be introduced once in every 3 to 7 years when no testing of either the source flock or the sheep in consignments was required. When only negative surveillance tests were required the interval increased to once in every 63 to 111 years and, with the additional requirement that all sheep in each consignment must have a negative test before and after transport, the interval further increased to once in every 125 to 333 years. When only sheep in consignments were tested, the interval was calculated to be 8 to 14 years. Controls on the importation of sheep were changed to provide an acceptable level of risk for the sheep industry in Western Australia.

Title The Victorian Ovine Johne's Disease Eradication Programme - Effect on Sheep Owners.

Author(s) Tobin FM.

Institution Victorian Ovine Johne's Disease Action Group, Winslow, Victoria, Australia.

Abstract In December 1996 the Department of Natural Resources and Environment commenced an eradication programme of Ovine Johne's Disease (OJD) in the state of Victoria, Australia. The programme was based on compulsory slaughter and destocking for a minimum of 2 summers of all sheep, deer, goats and alpaca on properties where OJD was identified. This paper describes the effects of this programme on sheep owners, their families and rural communities. The effects on farm profitability, the costs and logistics of changing to an alternative enterprise for the destocked period, the costs and problems faced in restocking the farm following the destocked period, and the hidden costs of eradication are examined. Effectiveness of a disease control programme such as this OJD control programme is clearly dependent on the cooperation of all sheep owners. The role of affected sheep owners and their cooperation in this control programme is discussed. This programme has provided a unique opportunity to evaluate the effects of an eradication programme which requires total destocking of all susceptible species for a minimum of 16 months.

Title Control of Ovine Johne's Disease in Victoria

Author(s) Millar HWC.

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Abstract Ovine Johne's disease (OJD) was diagnosed in a commercial fine wool merino flock in eastern Victoria in December 1995. Prior to this, there was no evidence indicating that sheep flocks in Victoria were infected. This paper presents an account of the tracing and investigation activities undertaken as a result of this diagnosis, and the disease control program which was initiated in partnership with the Victorian sheep industry in December 1996. Information on the number and outcomes of flock disease investigations is presented, and progress with the Victorian OJD control program discussed.

Title Vaccination does not prevent faecal shedding of Mycobacterium avium subsp. paratuberculosis

Author(s) Kalis CHJ, Hesselink JW, Barkema HW.

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Abstract In The Netherlands, experimental vaccination against paratuberculosis with a killed vaccine proved to be successful in prevention of clinical paratuberculosis in the early sixties but was prohibited at that time due to cross-reactions with tuberculosis tests. This experiment was repeated in the eighties after The Netherlands were officially free of bovine tuberculosis with comparable results: a positive cost-benefit of vaccination due to a decrease in the rate of clinical paratuberculosis (Kalis CHJ et al.). Nowadays attention is not only focused on the reduction of the economic damage due to clinical paratuberculosis but also on the eradication of the disease from the herds. So, the question arises if vaccination can lead to a decrease or even elimination of *Mycobacterium paratuberculosis* (Mptb) in a herd. It has been suggested that freedom of infection might occur after 4 to 6 years of vaccination (Argente G.). The purposes of the present study were: (1) to study if vaccination against paratuberculosis during at least 10 years prevents faecal shedding of Mptb; (2) to compare the effect of a culture and cull eradication program on faecal shedding of Mptb between vaccinated and not vaccinated herds. The total number of faecal samples in ten herds with a history of more than ten years of vaccination was 750. Of these samples, 37 (4.9%) were Mptb positive. In three herds (30%) no Mptb positive faecal sample was cultured. In the seven positive herds the prevalence varied from 1% to 29%. After a vaccination program of 12 years (5 of the 7 herds) the prevalence was 5.1%. In 39 herds with clinical paratuberculosis that never vaccinated against paratuberculosis a total number of 3045 faecal samples was cultured in the same period. Of these samples 182 were Mptb positive (6.0%). In seven herds (18%) no positive faecal sample was cultured. There was no significant difference between the vaccinated and the non-vaccinated herds in the rate of decline of culture-positives in the course of a two years culture and cull eradication program. In both groups the rate of faecal shedders declined over time with a sharp decline after the onset of the culture and cull program followed by a weak decline in the following period. This study demonstrates that a 10 years vaccination program is no guarantee that shedding of Mptb is prevented. Therefore a vaccination program has to be combined with, or replaced by a culture and cull program. The effect of a two year culture and cull program was not different between vaccinated and not vaccinated cows.

Title Compliance of Victorian Dairy Farmers with current calf rearing recommendations for the control of Johne's Disease.

Author(s) Wraight M, McNeil J, Beggs D, Greenall R, Humphris T, Irwin R, Jagoe S, Jemmeson A, Morgan W, Brightling P, Anderson G, Mansell P.

Institution University of Melbourne, Werribee, Australia.

Abstract Questionnaires were posted to 800 randomly selected registered Victorian dairy farmers in 1996. 534 responses were received and analysed. 13.2% of respondents stated that JD had been diagnosed on their farm in the last 5 years. JD was rated second only to neonatal diarrhoea in importance as a disease of calves, even though other diseases of calves occurred more frequently. However, there was a low level of compliance with JD control recommendations by the respondents. There was no significant difference in the number of JD control recommendations achieved between the three major Victorian dairying regions. There was a significant difference in compliance between farms having had a diagnosed case of JD and those that had not. Although there is awareness among dairy farmers of the importance of JD, there appears to have been a poor uptake by farmers of measures to prevent the spread of the disease. Current JD control recommendations and the method of information transfer should be reassessed to ensure that dairy heifers are reared with minimal risk of transmission of JD on Victorian dairy farms.

Title Genetic influence on the susceptibility of cattle to paratuberculosis.

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Abstract Both in tuberculosis and in leprosy there is clear evidence that genetic factors influence the susceptibility to infection. In the case of paratuberculosis no quantitative research has been performed with regard to this subject although this knowledge may be beneficial to control programs. We have analysed data, retrospectively, of a ten year (1984-1994) follow up study of the efficacy of vaccination against paratuberculosis in Dutch dairy cattle. Records of over 7000 animals from 20 different farms were processed using a standard polygenic model. Separate analyses were done on populations with significantly different prevalence dynamics in the 10 year period investigated as well as on vaccinated and non-vaccinated animals. A Bayesian analysis was performed to estimate the marginal posterior distribution of heritability by a Markov chain Monte Carlo algorithm, and flat priors were used for variance components. The results indicate that there is a small genetic influence on the susceptibility of cattle to become infected with *M. paratuberculosis*. Heritabilities ranged between 0.029 and 0.05, with genetic standard deviations between 0.068 and 0.086. These results appear to be typical for disease traits: significant genetic standard deviations, which indicate that there are interesting differences between animals, but with low heritability. Searching for genes responsible for these differences could be a next step, as traditional selection would be inefficient.

Title Use of DNA fingerprinting for epidemiological studies of bovine paratuberculosis in Sweden and Czech Republic.

Author(s) Bölske G¹, Pavlík I², Viske D³, Larsson B³, Englund S¹, Dvorska L², du Maine R², Parmova I⁴.

Institution ¹ National Veterinary Institute, Uppsala, Sweden, ² Veterinary Research Institute, Brno, Czech Republic, ³ Swedish Board of Agriculture, Jönköping, Sweden, ⁴ State Veterinary Diagnostic Institute, Prague, Czech Republic.

Abstract Due to trade liberalisation with farm animals, paratuberculosis is rapidly spreading among ruminants in many European countries. Two countries with a very different situation are Sweden and the Czech Republic. Sweden has a very low disease prevalence and a limited cattle import and the Czech Republic had a massive cattle import after 1992. Isolates of *M. paratuberculosis* from the two countries were examined using DNA fingerprinting (restriction endonucleases PstI and BstEII). Thirteen Swedish isolates of *M. paratuberculosis* from cattle were investigated. Five isolates from animals imported from Denmark (4 Blonde d'Aquitaine, 1 Limousine) and one isolate from a cow imported from Finland (Aberdeen Angus). Six isolates came from herds within a domestic chain of infection, in the Limousine breed, originating from import from France via Denmark in 1975. One isolate came from a Swedish Friesian cow, without any obvious connections to imported animals. All Swedish isolates were of DNA type B-C1. This supporting the infection spread from imported animals to animals originally reared in Sweden. Of 23 cattle herds originally reared in the Czech Republic, strains were of the DNA types B-C9 (6 herds), A-C10 (5 herds), D-C12 (8 herds), E-C1 (1 herd), and B-C1 (3 herds in contact with imported ruminants). In all 34 infected cattle herds (Blonde d'Aquitaine, Charolais, Holstein, Hereford, Jersey, Limousine, Mont Belliarde) imported from Denmark, France, Germany and Hungary, type B-C1 was the most prevalent. In three of these herds, one animal harboured a strain of another type (E-C1, A-C10 and I-C13). Among other ruminants with paratuberculosis, domestically reared sheep harboured types A-C10, A-C8, B-C1, B-C2, and E-C1, goats C-S1 and B-C1 and deer A-C10 and B-C1. In imported wild goats from Estonia type B-C1 was found, in imported goats from Denmark B-C1 and in deer from Scotland B-C16. Research was partially supported by the Ministry of Agriculture of the Czech Republic (grant no. EP0960006087) and the Swedish Council for Forestry and Agricultural Research (grant no. 31.1189/97).

Title Study of spreading *Mycobacterium avium* subsp. paratuberculosis of different DNA

fingerprints from farm to wild ruminants and survey performed in wild ruminants in the Czech Republic in the period 1995-1998.

Author(s) Pavlík I, Bartl J, Horvathova A, Dvorska L, Matlova L, Fischer O, du Maine R, Rozsypalova Z.

Institution Veterinary Research Institute, Brno, Czech Republic.

Abstract After 1989 paratuberculosis has been often diagnosed in imported ruminants. In December 1992, 19 highly pregnant heifers of the breed Charolais were purchased from Hungary (original import from France to Hungary). In January 1993 one heifer was born who left the herd in November 1993 and until May 1994 (ie. 7 months) moved freely in the nature in the range of 15 - 20 km. After having been caught, the clinical signs of paratuberculosis (emaciation and diarrhoea) were evident. Consequently, 4 other cases of animals infected with the same strain of DNA type B-C1 were revealed. Therefore in the period 1995-1996 sampling of the small intestine and corresponding lymph nodes from 83 shoot red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) from 43 different locations in the same district were examined. 6 strains of *Mycobacterium avium* subsp. paratuberculosis were totally isolated: one strain of DNA type B-C1 from a stag; four strains of DNA type B-C1 and one strain of DNA type B-C9 from roe deer. Regarding the fact that the five wild ruminants (one stag and four roe deer) infected with the strains of the DNA type B-C1 were located in the same area as the infected heifer, the infection source could be the infected heifer. In one roe deer infected with the strain of DNA type B-C9 the infection source was different: the stags who ran away from a farm were purchased from an area infected with this DNA type. In the following survey carried out during 1997 -1998 in the whole Czech Republic, divided into 74 districts, more than 400 heads of hoofed game were examined from more than 70 % of the districts. M.a.paratuberculosis has been found so far in 8 animals (stag, fallow deer, moufflon) kept in farms and game parks. In wild the infection has been so far found in two stags and a moufflon in the districts where infected cattle herds occurred. All the isolated strains are at present identified using DNA fingerprinting to assess all epizootiological relations. Our research was partially supported by the Ministry of Agriculture (grant no. EP0960006087) and the Ministry of Health of the Czech Republic (grant no. 4211-3/97). Permanent address of Robin du Maine - Hogeschool van Utrecht, Netherlands.

Title Investigation of false positives in the IS900 PCR for identification of *M. paratuberculosis*

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Abstract Johne's disease (JD), caused by *Mycobacterium paratuberculosis*, is a chronic enteritis and lymphadenitis affecting ruminants and camelids. JD is found in most countries in the world and is present in Australia. During surveillance work in Western Australia and test and cull procedures in Victoria, 4 isolates that were not Mycobactin dependent were found to be IS900 PCR positive. Restriction digest of the amplified products confirmed that the product was not consistent with the sequence of *M. paratuberculosis*. The isolates were further investigated using IS900 and 16S rRNA sequencing, and by restriction fragment length polymorphism (RFLP) using IS900 as probe. Sequence differences were detected between the two WA isolates but the two Victorian isolates were identical using 16S and IS900 sequencing. Using 16S sequencing, all isolates most closely resembled an unclassified IWGMT strain. Sequence differences were detected between the three strains within the amplified product of IS900 and homologies of between 73 and 83% were present within the IS900 target. In each case, sequencing confirmed that the selected restriction sites in these isolates were not consistent with *M. paratuberculosis*. Between 3 and 5 copies of the IS900-like sequences was confirmed by RFLP. Although it is accepted that there is homology at the 3' end of IS900 between *M. paratuberculosis* and other *M. avium* spp, the 5' end of this IS has previously been considered specific for *M. paratuberculosis*. The isolation and characterisation of these isolates has confirmed considerable homology with *M. paratuberculosis* in this region. The finding of these organisms suggests that IS900 PCR may be less than 100% specific and it is suggested that where such technology is used for identification of *M. paratuberculosis*, a restriction digest be applied to confirm the internal sequence of the amplified product.

Title Study of IS900 loci in *Mycobacterium avium* subsp. *paratuberculosis* by multiplex PCR screening.

Author(s) Bull T, Pavlík I¹, Hermon-Taylor J, Tizard M.

Institution Department of Surgery, St. George's Hospital Medical School, Cranmer Terrace, London, UK. ¹ Veterinary Research Institute, Brno, Czech Republic.

Abstract We have used a *Mycobacterium avium* subsp. *paratuberculosis* (MAP), strain Linda, genomic library to select clones containing copies of IS900 and to sequence their flanking regions. This approach has determined sequences from 13 of the 16 loci predicted for this strain by RFLP analysis. 12 of these loci are located immediately upstream of ORFs inside a putative ribosome binding motif AGGAGA. Analysis of homologous genomic regions in *Mycobacterium avium* subsp. *avium*, indicates that IS900 insertion effects deletion of the terminal base to this motif. The relative orientation of IS900 with these ORFs is consistent with the transcription of *hed*. Only one locus contained an IS900 insertion inside an ORF the orientation of which was consistent with the transcription of *p43*. Using these data we have developed a rapid PCR multiplex typing method based upon the presence/absence of IS900 in these loci. This method uses an anchor primer designed from the 3' end of IS900 in conjunction with 13 locus specific primers. These are designed to give PCR products that differ by at least 50bp, visualised as a PCR product 'ladder' on 1.5% agarose electrophoresis gels. IS900 loci that are not filled or have undergone genomic re-arrangements relative to the Linda strain, do not produce PCR products. We have screened over 50 strains of MAP using this system, including representatives of all previously published RFLP types from environmental, sheep, cattle, goat, primate and human sources. Results show that the multiplex system is not as variable as RFLP. However consistent differences were seen between IS900 loci in sheep strains and MAP from other sources, probably due to genomic re-arrangements. Unique multiplex profiles were detected in some strains that had identical RFLP patterns. In addition, isolates from two human isolates and one

primate isolate gave a unique multiplex profile.

- Title** Strain typing of *M. avium* subsp. paratuberculosis and *M. avium* based on polymorphisms in IS1311.
- Author(s)** Marsh I¹, Whittington R¹, Cousins D².
- Institution** ¹ Elizabeth Macarthur Agricultural Institute, NSW Agriculture, Menangle, New South Wales, Australia, and ² Animal Health Laboratories, Agriculture Western Australia, Perth, Western Australia, Australia
- Abstract** Eradication and control programs of Johne's disease (JD) in Australia assume that cattle are not susceptible to infection with the sheep strain of *M. avium* subsp. paratuberculosis and can safely graze on pasture with or after the removal of sheep affected with ovine Johne's disease. Therefore, ongoing strain identification of JD cases is essential to substantiate this assumption. Currently diagnosis of JD is confirmed by Polymerase chain reaction (PCR) on IS900, an insertion sequence (IS) unique to *M. avium* subsp. paratuberculosis and strain identification is achieved by restriction fragment length polymorphism (RFLP) analysis, an expensive and time consuming process. Recently, another insertion sequence, IS1311, was found in *M. avium* subsp. paratuberculosis and *M. avium*. Characterisation of IS1311 in sheep and cattle strains of *M. avium* subsp. paratuberculosis revealed several point mutations compared to the *M. avium* sequence and these can be used to discriminate between the species. In addition, a polymorphic IS1311 locus in the cattle strain of *M. avium* subsp. paratuberculosis can be used to differentiate it from the sheep strain. A rapid and sensitive PCR- test based on IS1311 has been developed and validated on an extensive range of *M. avium* subsp. paratuberculosis and *M. avium* isolates from a variety of sources including primary radiometric cultures, purified DNA and crude DNA from cultured organisms. With this test we confirmed the presence of *M. avium* subsp. paratuberculosis or *M. avium* and achieved a 100% correlation with RFLP or species of origin.

- Title** A low G+C content element in *Mycobacterium avium* subsp. paratuberculosis and *M. avium* subsp. silvaticum with homologous genes in *M. tuberculosis*.
- Author(s)** Tizard M, Bull T, Millar D, Doran T, Martin H, Ford J, Hermon-Taylor J.
- Institution** Department of Surgery, St. George's Hospital Medical School, Cranmer Terrace, Tooting, London, UK.
- Abstract** The genetic subtraction technique known as representation difference analysis (RDA) PCR was applied to look for DNA and genes specific to *Mycobacterium avium* subsp. paratuberculosis. This generated a 671 bp DNA fragment that was used to isolate a larger genetic element found in *M. avium* subsp. paratuberculosis and *M. avium* subsp. silvaticum, both associated with enteric disease. This element, designated GS, was absent from the very closely related and relatively benign *M. avium* subsp. avium. It is more than 6.5 kbp in length and has a G+C content 9 % lower than other genes from this species indicating that it originates from an organism outside of the *M. avium* complex (MAC). A previously uncharacterized insertion sequence is associated with one end. The GS element encodes five ORFs, all of which have counterparts encoded in *M. tuberculosis*. and a number of bacterial species predominantly Gram negative organisms, including a number of enteric pathogens. Genes homologous to those found within GS encode functions related to the biosynthesis of LPS or extracellular polysaccharide. This element has a number of features in common with pathogenicity islands particularly its low G+C content, also its absence from a less virulent close genetic relative, *M. avium*, its association with an insertion sequence and the grouping of genes of related function with putative virulence linkage. No direct link to pathogenicity has been shown but GS may belong to a group of related 'genetic islands' and represents the first mobile genetic element of this class to be identified in mycobacteria.

- Title** Further studies on the GS element: A novel mycobacterial Insertion Sequence (IS1612), inserted into an acetylase gene (*mpa*) in *Mycobacterium avium* subsp.

icum but not in *Mycobacterium avium* subsp. *paratuberculosis*.

Author(s) Bull T, Martin H, Sumar N, Tizard M, Hermon-Taylor J.

Institution Department of Surgery, St. George's Hospital Medical School, Cranmer Terrace, London, UK.

Abstract We have recently described the GS element, found in *Mycobacterium avium* subsp. *paratuberculosis* (MAP), *Mycobacterium avium* subsp. *silvaticum* (MAS) and some isolates of *Mycobacterium avium* subsp. *avium* serotype 2 (MAAs2), which contains a set of genes of low GC% content, possibly associated with the biosynthesis, modification and transference of fucose to cell wall glycopeptidolipids. Here we describe a further gene of low GC% content (*mpa*), immediately downstream of the GS element. *mpa* is a putative acetylase with homology to genes directly responsible for host specificity and virulence in *Salmonella typhimurium* and *Shigella flexneri*. Unlike other GS genes, homologues of *mpa* have not been found in related species, including *Mycobacterium tuberculosis* (MTB). In MAP, *mpa* encodes an ORF of 445aa, however in MAS and MAAs2 it contains a single inserted copy of a novel insertion sequence. This element (IS1612) has two sets of inverted repeats at each terminus and encodes two ORFs with good homologies to transposase and helper proteins of IS21 (*E. coli*) and IS1415 (*R. erythropolis*). Sequence comparisons between *mpa* in MAP and MAS indicate the target site for IS1612 is duplicated on insertion to give a direct repeat at each end of the element. Immediately downstream of the *mpa* gene in both MAP and MAS are a group of three genes with good homology to the Daunorubicin resistance cluster. This cluster has a high GC% content which suggests a "border" for the GS element. A short motif present at the beginning of this cluster matches with and inverted repeat of this motif at the beginning of the first gene in the GS element. This encapsulates the whole of this group of low GC% genes in MAP and further suggests its cassette-like nature. Homologues of the GS element in MTB show a marked similarity of organisation, suggesting a parallel role for these genes in both pathogens.

Title Expression, Purification and Seroreactivity of the GS proteins of *Mycobacterium avium* subsp. *paratuberculosis*.

Author(s) Martin HM, Sumar N, Bull TJ, Tizard ML, Hermon-Taylor J.

Institution Department of Surgery, St George's Hospital Medical School, London, SW17 ORE, U.K.

Abstract We are investigating a series of 6 contiguous open reading frames, (*gsa*, *gsbA*, *gsbB*, *gsc*, *gsd* and *mpa*), isolated from a low G+C content genetic element in *M. avium* subsp. *paratuberculosis*, designated GS1. Analysis of DNA and protein databases show that components of the GS element, which possess homologues in the pathogen *M. tuberculosis* but not *M. bovis* BCG, may encode functions related to extracellular polysaccharide synthesis or modification, and as such may influence the pathogenicity of *M. avium* subsp. *paratuberculosis*. We have designed constructs which facilitate the expression of the GS genes in bacterial and insect cell expression systems and the purification of the recombinant proteins utilising metal chelate chromatography. Purification of the GS components from *E. coli* and SF9 insect cells will provide reagents to test the diagnostic capability of the GS element. The reactivity of purified recombinant GS proteins with sera from Johne's disease cattle, Crohn's disease patients and appropriate controls can then be evaluated. In addition, GS specific anti-peptide rabbit polyclonal antibodies have been produced for use as reagents for further analysis of function of the GS element. 1 A low G+C content genetic island in *Mycobacterium avium* subsp. *paratuberculosis* and *M. avium* subsp. *silvaticum* with homologous genes in *M. tuberculosis*.

Title Homogeneity/heterogeneity of strains of *Mycobacterium avium* subsp. *paratuberculosis*: suggestion for the standardisation of methods, analysis of worldwide results published from 1990-1998, and the correlation between DNA-type of a strain and its origin (animals, environment, man).

Author(s) Pavlík I, Horvathova A, Dvorska L, Svastova P, du Maine R, Fixa B¹, Rychlik I.

Institution Veterinary Research Institute, Brno, Czech Republic. ¹ II. Clinic of Internal Medicine, Faculty Hospital, Charles University, Hradec Kralove, Czech Republic.

Abstract We have recently suggested a standardisation of the method for fingerprinting *Mycobacterium avium* subsp. *paratuberculosis* using IS900 and restriction endonucleases PstI and BstEII. In 1997, based on a study of 620 strains, 11 RFLP types were detected after digestion with restriction endonuclease PstI, (designated as A - K) and 15 RFLP types after digestion with restriction endonuclease BstEII (designated as C1-3, C5, C7-17, S1, and I1). After a parallel examination of DNA from individual strains, a total of 23 different RFLP types were detected. These results were complemented by another 140 strains in 1998. DNA fingerprints were scanned by CCD camera and analysed by software Gel Manager (Biosystematika, Tavistock, UK). A total of 740 strains of *M.a.paratuberculosis* from 10 different species of animals (cattle, sheep, goat, rhinoceros, wild goat, moufflon, fallow deer, roe deer, deer, etc.) from 25 laboratories and 23 countries were analysed. In addition 12 strains isolated from patients with Crohn's disease and 20 strains from the environment were included. From a total of 23 RFLP types, in Europe (13 countries) 15 RFLP types were identified (65.2%), in Australia and New Zealand 6 RFLP types (26.1%), in the USA, 7 RFLP types (30.4%). B-C1 was the most common RFLP type in the 3 continents, which was also found in 13 European countries. The second was RFLP type A-C10, identified in four European countries, the third was RFLP type E-C1, found only in three European countries. 17 RFLP types were identified in cattle, 9 RFLP types in sheep, 5 RFLP types in goats, three RFLP types in wild ruminants. Strains isolated from patients with Crohn's disease were classified to 4 RFLP types. Our research was partially supported by the Ministry of Agriculture (grant no. EP0960006087) and the Ministry of Health of the Czech Republic (grant no. 4211-3/97). Permanent address of Robin du Maine - Hogeschool van Utrecht, Netherlands.

Title Comparison of culture of *Mycobacterium paratuberculosis* in individual and strategically pooled bovine faecal samples

Author(s) Kalis CHJ, Hesselink JW, Barkema HW.

Institution Department of Ruminant Health, Animal Health Service, PO Box 361, 9200 AJ Drachten, The Netherlands.

Abstract A modified procedure (Kalis et al 1998) for culture of *Mycobacterium paratuberculosis* (Mptb) from bovine faeces, based on the method of Jørgensen for faecal samples from individual cows was used for the culture of pooled faecal samples from five cows each. In eleven dairy herds faecal samples from 733 cows were cultured both individually and pooled. Pooling of samples was performed age depending (strategically). Individual cultures demonstrated the presence of Mptb in six from these eleven herds, and in 43 from these 733 cows. The pooled faecal cultures detected Mptb in seven out of eleven herds and in 28 out of 151 pools. Six culture positive animals were not detected in pooled faecal cultures but on the other hand two pools in which no positive animals were detected by individual culture, were culture positive. If compared with individual culture as golden standard, sensitivity from the pooled faecal culture method was 86 % and specificity was 96 %. The average number of colonies in positive cultures of pooled faecal samples was 22 and the total number of colonies in the five corresponding individual cultures was 31. The high sensitivity of this pooled faecal culture method was unexpected because experiments with pooled faecal culture based on another culture method reported a sensitivity of only 38%, compared with individual faecal cultures (Vialard et al 1993). This decrease of the total number of colonies after pooling the samples was lower than expected because mathematically this decrease was expected to be 80% instead of 29% in pools containing only one positive sample. The conclusions were: (1) the effect of pooling of faecal samples on sensitivity of the method cannot be estimated without taking into account the method of culture and the method of pooling. (2) culture of strategically pooled faecal samples with the modified Jørgensen method proved to be a good alternative for culture of individual faecal samples to detect the presence of Mptb in dairy herds and leads to a significant reduction of costs and (3) the difference between the sensitivity of the described culture method and the method reported earlier in pooled faecal samples cannot be explained and further research is needed before a general advice about pooling can be given.

Title Use of culture and serology in tracing and screening for paratuberculosis in Swedish cattle.

Author(s) Bölske G¹, Viske D², Larsson B², Sternberg S¹.

Institution ¹ National Veterinary Institute, Uppsala and ² Swedish Board of Agriculture, Jönköping, Sweden.

Abstract After the finding of paratuberculosis in imported cattle in Sweden in 1993, an increased number of diagnostic tests have been performed. In a survey, serology with an absorbed ELISA and faecal culture were performed on each imported animal. A large number of tests were also carried out for tracing on and back from known infected herds. Cattle with serological reactions in ELISA were tested with faecal culture or sent to slaughter and cultured from ileum and the ileocaecal lymph node. When *Mycobacterium paratuberculosis* was isolated from an animal, the whole herd was stamped out and ileum and ileocaecal lymph nodes were collected from a number of cattle at the abattoir for culture and histopathological examination. During 1993 - 1997 approximately 6000 animals were cultured and 10600 blood samples were tested in ELISA. *M. paratuberculosis* was isolated from 107 animals in 44 herds. Seventy-five of these animals were also tested serologically, 11 of which proved ELISA positive. Blood samples from a total of 132 animals were positive in ELISA. Forty-six of these animals were slaughtered and samples from ileum and the ileocaecal lymph node were cultured and histopathologically examined. *M. paratuberculosis* was isolated from 11 of these cases. Histopathological lesions were only found in cases with a strong serological reaction. The apparently low sensitivity of the absorbed ELISA in our situation may be explained by an early stage of infection in most cases. Our observations illustrate the difficulties in detecting early cases of paratuberculosis with the absorbed ELISA and the problem of optimising the use of diagnostic techniques in the

tracing of infected animals.

Title ELISA and faecal culture: Sensitivity and specificity of each method.

Author(s) Whitlock RH, Wells S, Sweeney RW, Van Tiem J.

Institution University of Pennsylvania, New Bolton Center, Kennett Square, PA, USDA/APHIS/NAHMS, Fort Collins, CO; & USDA/APHIS Riverdale, MD.

Abstract This presentation will review the published reports of sensitivity and specificity of the CSL and IDEXX ELISA test for paratuberculosis in cattle. Additional ELISA and faecal culture data from twenty dairy herds where both faecal cultures and ELISA testing was done concurrently will be included. A cohort of 954 cattle cultured every six months from ten herds followed over four years will serve as the basis to ascertain the sensitivity of faecal culture to detect infected cattle in dairy herds. The cohort of 954 cattle included a cohort of adult (lactating) cattle of 697 cattle which were also sampled over the same four period at six month intervals. The sensitivity of faecal culture was based on state of the art culture techniques including centrifugation and double incubation. Of the 954 cattle cohort of all ages (calf to adult) that were faecal samples on the first herd visit, 79 were culture positive. An additional 131 animals were detected as culture positive over the next 3 1/2 years, when cultured at six month intervals. The sensitivity of faecal culture to detect infected cattle on the first sampling was 38%. With the 697 adult cattle cohort, 67 were positive on the first faecal culture, while an additional 91 were culture positive over the next 3 1/2 years, giving a sensitivity of detection at first culture of 42%. In neither case does this consider the animals culled from the herds prior to being detected, nor does it include the animals always culture negative but that will have culture positive tissues at slaughter. Both of these considerations will lower the apparent sensitivity of faecal culture.

Title Comparative sensitivity of ELISA and various faecal culture methods in dairy cattle herds with endemic Johne's disease.

Author(s) Eamens GJ, Turner MJ, Whittington RJ, Marsh IB, Saunders V, Kemsley PD, Rayward D.

Institution Elizabeth Macarthur Agricultural Institute, Camden, Australia.

Abstract In three New South Wales dairy cattle herds with endemic Johne's disease, prevalence rates by faecal culture were determined to be 11%, 18% and 19% respectively. Whole herd faecal culture was shown to detect markedly more infected cattle than whole herd testing by the EMAI absorbed ELISA, particularly in the two herds with greatest prevalence. In the three study herds, five methods for whole herd faecal culture were compared in each. These included two methods based on primary culture on Herrold's egg yolk medium with Mycobactin J (HEYM): (1) conventional decontamination with sedimentation and primary culture on HEYM; (2) Whitlock decontamination and culture on HEYM. The remaining three methods were based on radiometric (BACTEC) culture: (3) decontamination and filtration to BACTEC medium; (4) modified Whitlock decontamination to BACTEC medium and (5) Whitlock decontamination to BACTEC medium. For BACTEC cultures, two methods were compared as confirmatory tests for *M. paratuberculosis*: mycobactin dependence on conventional subculture to HEYM and IS900 PCR analysis of radiometric media. Among 179 cattle tested simultaneously by all 5 culture methods, 38 cattle were confirmed to be shedding *M. paratuberculosis* and a further 3 were suspected to be shedding *M. paratuberculosis*. In identifying shedder cattle, method 5 was the most sensitive, followed by methods 2, 4, 1 and 3 was the least sensitive. The number of BACTEC cultures confirmed to contain *M. paratuberculosis* was similar for the two methods used. Reliability of diagnostic methods (clinical examination, faecal culture, allergic and serological tests) in paratuberculosis of cattle and sheep during the eradication and control programme from 1988-1998.

Title Reliability of diagnostic methods (clinical examination, faecal culture, allergic and serological tests) in paratuberculosis of cattle and sheep during the eradication and control programme from 1988-1998.

Author(s) Pavlík I, Vesely T, Bartl J, Horvathova A, Matlova L, Valent L¹, Miskovic P², Hirko M².

Institution Veterinary Research Institute, Brno, Czech Rep. ¹ Regional Veterinary Administrations, Czech Rep. and Slovakia. ² Field veterinarian of relevant farm.

Abstract We have performed intensive research in recent years into paratuberculosis infection within our herds of ruminants. This experience, together with diagnostic testing has been used to a farm control programme performed for more than 3,000 cows on more than 15 farms and 400 sheep on one farm. The results of sensitivity and specificity have been obtained based on culture examinations of 21,148 faecal samples and laboratory tests of organs from 1,943 cattle and 370 sheep. Clinical signs such as diarrhoea and weight loss were seen in only 5 to 15% of the infected animals. *Mycobacterium avium* subsp. paratuberculosis was detected in 68.8% of cattle with diarrhoea and weight loss, and in 50% of sheep with weight loss. The occurrence of these clinical signs were more frequent, especially during the feed-transition from winter to summer and also after parturition. Clinical disease occurred most often in cattle aged 2 to 6 years. Using allergenodiagnosis 26.8% sensitivity at 72.0% specificity were obtained using avian tuberculin and 22.2% and 67.7% using Johnin. Examination of 3,026 allergologically reacting animals from infection-free herds showed a 99.4% AGID specificity, and 762 animals examined by ELISA showed a 93.3% specificity. Sensitivity of these methods and CFT was dependent on the level of animal infection. In the course of the development of the disease, the sensitivity of the examinations by AGID, CFT and ELISA increased from 18-20% to 80-90%. In clinically healthy animals with confirmed excretion (low shedders), the disease could not be serological diagnosed in more than 85% of animals by AGID, CFT and ELISA, also sensitivity correlated to the extent of excretion. Faecal cultures were negative in 25.0% animals with positive findings in mesenteric lymphatic nodes and intestinal mucous.

Title Comparison of the CSL and IDEXX Assays for Detection of Antibody to the Johne's agent: Studies on Reproducibility and Correlation with Faecal Culture Results.

Author(s) Jacobson RH, Byrum B, Whitlock RH, Stabel JR.

Institution Cornell University, Ithaca, New York; Ohio Department of Agriculture, Reynoldsburg, Ohio; University of Pennsylvania, Kennett Square, Pennsylvania; National Animal Disease Center, Ames, Iowa.

Abstract CSL and IDEXX test kits for antibody to *Mycobacterium paratuberculosis* were compared for their relative reproducibility and correlation of test results with faecal culture analysis. A panel of 10 standard samples was tested in each plate to compare between-run variation of the two kits. Also, each lab chose 108 unique sera, with 1/3 of them tested in each run to compare between-duplicate variation and agreement with faecal status for the two test kits. The median coefficient of variation (CV) for duplicates of the 108 samples run in each lab on the CSL kit was 4.3%, 3.8%, 9.9% and 20.0% for the 4 labs, respectively (mean = 7.1%). The comparable data for the IDEXX test kit was 3.1%, 2.6%, 4.9% and 3.2% (mean = 3.5%). The average CV for three runs of the 10 standard samples run in the CSL assay was 2.0%, 3.8% and 9.8% for three of the labs (average of 5.2%), while the comparable data on the IDEXX assay was 27.6%, 4.1%, and 21.8% (average of 17.8%). (The fourth lab inadvertently tested all samples in one 3-plate run using only 1 set of standards for the CSL kit so CV's for standards could not be calculated for comparison with the IDEXX kit data). Calculation of ratios to normalise the IDEXX results, as per kit instructions, increased the average for the median CVs of the three labs to about 25.7% (outliers eliminated to reduce bias). Antibody test results were discrepant between the CSL and IDEXX assays for 90 of the 481 samples (20.7%). Faecal culture status agreed with both assays for 62.1%-12.4% (SD) of the animals tested. Faecal culture status agreed with antibody test results slightly more often for the CSL assay (74.0%-7.0%) than for the IDEXX assay (71.0%-11.9%). The major difference between kits was the between-run variation in the IDEXX kit using normalised data (ratios as specified in kit instructions); it was about 3.4 times greater than the CSL non-normalised ODs - an unexpected result. Such differences indicate the need for a more in-depth study to isolate and rectify the source(s) of error that may affect serological classification of animal infection status.

Title Automation of an Absorbed Enzyme Immunoassay for the Detection of Mycobacterium paratuberculosis Antibodies for an Eradication Programme.

Author(s) Dimech W.

Institution Victorian Veterinary Pathology Services, South Yarra, Australia.

Abstract As part of an ongoing Johnes Disease eradication programme by the state of Victoria, Australia, the Victorian Veterinary Pathology Services (VVPS) has been involved in the testing of over 150,000 cattle samples per year for the presence of Johnes-specific antibodies. The PARACHEK® kit(CSL, Victoria, Australia) has been used throughout the project. The operational challenge was to incorporate the testing strategy within a busy Medical Pathology Practice and utilise some of their automation and process designs to produce an efficient and cost-effective testing strategy . Up to 3,000 tests are analysed daily during the peak season. This presentation details the work processes that have been developed over the past two years. Automation is used to both pipette and analyse samples. The Rosys (Dade/ Behring Diagnostics), a four-probe pipetting station is used to aliquot samples and absorbing diluent into microtubes. A Tecan pipette (ICN Biomedicals) is used as a supplementary workstation. All EIA plates are analysed using the Behring ELISA Processor 3 (Dade/ Behring Diagnostics), an automated instrument which washes EIA plates, adds reagents, reads absorbances as well as performs the data reduction of the results. Using automation has saved about 15 hours of labour per day. Problems that were encountered include pipetting issues, the kinetic end-point of the Paracheck, and excessive packaging of the kit. Maintenance, calibration and control of the analysers have become an integral part of the process. Application of innovative technological advances, especially in information technology is seen as the major next step. The flow of information such as laboratory numbers, animal identification and results of initial and repeat testing involves a considerable time. The use of information technology to control this function has the added benefit of allowing the testing institution to retrieve meaningful and complex data more easily. VVPS has also been working in conjunction with CSL to modify the PARACHEK® kit to make automation of the product more convenient. The approach includes a timed reaction end-point, a reduction in packaging and a change in reagent volume.

Title Evaluation of Johne's disease ELISA testing in north Queensland cattle and its application in proving 'Freedom' in Queensland, an historically free zone.

Author(s) Pitt DJ.

Institution Oonoonba Veterinary Laboratory, Townsville Australia.

Abstract Four hundred and seventy five blood samples from 18 high risk dairy herds on the Atherton Tablelands and five hundred and forty one blood samples from 16 conveniently sampled beef herds in north Queensland were collected and JD ELISA tested between July 1995 and October 1996. The objective was to evaluate the CSL JD ELISA test with regard to specificity and suitability for export certification and active surveillance in north Queensland. Following histopathology and culture of reactors we established a beef cattle specificity of 98.5 % (95% CI 97.1% to 99.3%) and a dairy cattle specificity of 98.3% (95% CI 96.7% to 99.3%). Active surveillance testing from March to December 1997 was undertaken in Queensland cattle. Eighty nine high risk dairy herds were selected and all animals over 4 years of age blood sampled. High risk herds were those which had imported animals from southern states, had a history of scouring or had unexplained deaths in mature animals. Seven thousand, seven hundred and fifty five dairy cows were ELISA tested and those with reactors were cultured for JD on 2 occasions 3 months apart. Dung and blood samples were collected from beef herds enrolled as part of active animal health surveillance. These properties were randomly selected from all cattle shires with approximately 30 mature animals tested from 154 herds. Animals that reacted to the ELISA, had a dung sample cultured. The project resulted in a beef cattle specificity of 95.5% (95% CI 94.9 to 96.1%) and a dairy cattle specificity of 97.7% (95% CI 97.3% to 98.0%). The evaluation and application in active surveillance will be further outlined and results of culturing with regard to other mycobacteria sp. (not M. paratuberculosis) will be discussed.

Title Performance of an absorbed ELISA in the detection of Johne's disease in sheep flocks in New South Wales, Australia.

Author(s) Marshall DJ¹, Kearns C¹, Whittington RJ², Eamens GJ², Manchester PE¹.

Institution New South Wales Agriculture, ¹ Regional Veterinary Laboratory, Orange, 2800, NSW, Australia; and, ² Elizabeth Macarthur Agricultural Institute, Menangle, 2568, NSW, Australia.

Abstract Industry and government representatives have recently agreed to a National program to control the spread of Johne's disease throughout sheep flocks within Australia. Reliable flock diagnostic tests will play an important role in detecting infected flocks and subsequent control of the disease. Throughout Australia the Agar Gel Immunodiffusion test (AGID) is the serological test currently used for diagnosis of Johne's disease in sheep. With the aim of developing a better serological test we have compared the performance of an absorbed ELISA with that of the AGID on a large bank of sera collected from sheep of known Johne's disease status. For studies of sensitivity, sera (n=290) were obtained from merino sheep from within known infected flocks. The presence of disease was confirmed by histology on a single 5cm piece of terminal ileum collected when the flock was slaughtered. Identification of infected sheep by this method allowed sera to be collected from a range of clinically and subclinically affected sheep. Sera used for specificity studies (n=1748) were obtained from Merino sheep originating from properties believed to be free of ovine Johne's disease based on history and clinical signs. ELISA results were expressed as ELISA Ratio (ER) being the measure of the reactivity of the test serum in relation to a negative control. The performance of the AGID and ELISA were comparable (sensitivity 32%, specificity 99%) when the ER cut-off was set at 3.6. Higher ELISA sensitivity (50%) could be achieved at the expense of specificity (95%) if an ER cut-off of 2.4 was used.

Title Performance of diagnostic tests to detect ovine Johnes disease.

Author(s) Condron RJ, Hope AF, Kluver PF, Jones S¹, Millar HWC².

Institution Victorian Institute of Animal Science Attwood; ¹ CSL Ltd., Poplar Road, Parkville; ² Office of the Chief Veterinary Officer, Department of Natural Resources and Environment, Victoria, Australia.

Abstract Following the detection of Johnes disease in sheep flocks in Victoria an eradication strategy was implemented on infected farms and an intensive tracing and surveillance program was initiated. In order to assess the performance of diagnostic tests for ovine Johnes disease specimens from 500 sheep from five farms were examined histologically and tested by agar gel immunodiffusion (AGID) and by absorbed EIA (PARACHEK®). The sheep were selected so that 80% were representative of the mob and 20% were from the 'tail' of the flocks with a body condition score < 2. Three of the flocks were selected on the basis of high prevalence of clinical disease and two with low prevalence of clinical disease. Infection was confirmed in 25% of the sheep with the prevalence in the 'tail' 2 to 8 times the prevalence in the mobs. The AGID had a sensitivity of 74% (37/50) for infected sheep in the 'tail' and overall sensitivity of 46.7% (56/120). To assess the specificity of PARACHEK® sera from 1000 Merino sheep from Western Australia which is not known to have Johnes disease were tested. Various cut-off algorithms were used to best determine the most appropriate for field use. By modifying the cut-off algorithm for cattle to negative control OD plus 0.200, the specificity was 99.3 ± 0.5%. Using the modified cut-off calculation PARACHEK® detected 44.2% (53/120) of infected sheep which is not significantly different from AGID. Both serological tests were effective in detecting infected sheep. In the mob with the lowest prevalence (2%) PARACHEK® was positive for one infected animal but the AGID was negative for both infected animals. Although all sheep were in poor condition due to limited feed following drought conditions, sheep with the lowest body score had a higher prevalence of Johnes disease. Sampling sheep with lowest body score enhances the sensitivity of serological detection of infection.

Title A New Assessment of the Absorbed ELISA for Diagnosis of Sheep Paratuberculosis

Author(s) Garrido JM¹, Aduriz G, García Marín JF², Pérez V², Corpa JM, Juste RA¹.

Institution ¹ NEIKER (Instituto Vasco de Investigación y Desarrollo Agrario) (SIMA), Berreaga, 1. 48160 Derio, Bizkaia. SPAIN ² Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, 24007 León. SPAIN

Abstract Diagnosis of sheep paratuberculosis is difficult because of the low efficiency and high costs of culture and inconvenience of serological methods: lack of sensitivity of AGID, and excess of nonspecific reactions in the ELISA. In order to reduce the background noise in the absorbed ELISA we assayed a series of modifications in the protocol essentially based in the use of different conjugates. Three sera of different levels of reaction intensity were used to compare the signal to noise ratio of different conjugates at different concentrations. Once the best combination was chosen, the protocol was tested on a sample of 118 sheep of known paratuberculosis status. When only culture results were considered, the modified ELISA showed a sensitivity of 100%, and an specificity of 77.0%. If only histopathological results were taken as the reference the specificity was 89.1% and the sensitivity 79.2%. When a combination of both techniques was used as the gold standard, sensitivity and specificity reached 96.2% and 100%, respectively.

Title The Histopathologic Diagnosis Of Early Johne's Disease

Author(s) Buergelt CD, Ginn PE.

Institution Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA

Abstract Preferred tests for the diagnosis of Johne's disease are divided into two major categories: (i) agent detection; (ii) specific serum antibody/cell-mediated immunity detection. Agent detection tests include bacterial culture, genetic probes and biopsy. Many of the presently used tests suffer from inability to target early infection with Mycobacterium paratuberculosis. Early detection of Johne's disease by biopsy of intestine and/or draining mesenteric lymph nodes is based on the observation of inflammatory changes and/or acid-fast bacilli resembling Mycobacterium paratuberculosis in target tissues. Pathognomonic cellular changes include clustered epithelioid macrophages and/or individual or several inflammatory giant cells of Langhans' type (L type). These cells may have phagocytized one or more acid-fast bacilli. The ideal tissue to harvest is mesenteric lymph node. The most significant hallmark for early diagnosis is the Langhans' type giant cell located in the paracortical zone of the mesenteric lymph node. Pathologists are divided as to the criteria needed for a confirmatory diagnosis of early Johne's disease. Uniformly accepted guidelines for the positive diagnosis should be established and adhered to by pathologists. There is a void not only for uniformity of interpretation, but also for establishing sensitivity and specificity for the biopsy as diagnostic tool. The tissue obtained through biopsy can be additionally used for immunohistochemistry, genetic probes and culture of infectious bacilli. In conjunction with the herd history the biopsy has potential usefulness as a diagnostic test for screening individual animals under consideration for addition to paratuberculosis negative collections of zoo artiodactyla or cattle herds with infection negative status. Despite the cost and labour-intensity (laparotomy) the biopsy could become a valuable test for the control of paratuberculosis. This presentation will demonstrate histopathologic examples of early Johne's disease and pitfalls of interpretation.

Title Evaluation of the MGIT system for culturing Mycobacterium paratuberculosis and characterisation of strains by polymerase chain reaction tests.

Author(s) de Lisle GW, Yates GF, Cavaignac S, Collins DM.

Institution AgResearch, Wallaceville Animal Research Centre, P.O. Box 40-063, Upper Hutt, New Zealand.

Abstract Previous studies using DNA fingerprinting revealed three major groups of M. paratuberculosis. One of these groups, which in New Zealand is found principally in small ruminants and deer, is

not readily isolated in primary culture on Herrold's egg yolk medium supplemented with mycobactin. In this study we evaluated the use of a liquid culture system (MGIT, Becton Dickinson) modified by the addition of mycobactin, for isolating *M. paratuberculosis* from lymph nodes of deer and sheep. A total of 85 frozen homogenates of tissues known to contain mycobacteria were cultured using MGITs and Herrold's egg yolk medium. The identity of isolates was confirmed as *M. paratuberculosis* using a PCR test based on the insertion element IS900. Further characterisation of isolates was carried out using a PCR test based on primers from IS900 which generated a PCR product with isolates from the "ovine" DNA group of *M. paratuberculosis* but not from the "bovine" DNA group. Growth of *M. paratuberculosis* was observed in the MGIT system after 3 to 37 days incubation in 17 of 18 samples containing large numbers of mycobacteria. In contrast, growth on Herrold's medium for these samples took a minimum of 21 days and only 3 of 18 had grown on the solid medium by 37 days. Examination of the isolates by PCR indicated that members of the "bovine" DNA group can be readily isolated from tissue samples using the MGIT system. Furthermore, some members of the "ovine" group can also be isolated from tissues using MGITs.

Title Identification of *Mycobacterium paratuberculosis* by IS900 PCR after faecal culture on Lowenstein-Jenssen medium in the presence of mycobactin.

Author(s) Van Maanen C¹, Jarings G¹, Visser IJR¹, Willemsen PTJ², Bakker D².

Institution ¹ Animal Health Service, The Netherlands. ² ID-DLO, The Netherlands

Abstract At the Animal Health Service in the Netherlands faecal culture for *M. paratuberculosis* is performed on a relatively large scale. Routinely, faecal samples are decontaminated, and subsequently cultured on Lowenstein-Jenssen medium in the presence of mycobactin. Cultures are inspected every four weeks for up to 26. When suspected colonies were present, a Ziehl-Neelsen stain is performed. Ziehl-Neelsen positive cultures are at the moment confirmed using the IS900 PCR. We evaluated the confirmation results on 426 Ziehl-Neelsen positive cultures using the IS900 PCR. Only one out of 426 PCR reactions was negative without amplification of the internal control, apparently due to inhibition. However, 69 out of 425 (16 %) Ziehl-Neelsen positive cultures were negative in the IS900 PCR, whereas 356 Ziehl-Neelsen positive cultures were positive in the IS900 PCR. Subsequently, the mycobacterial status of 32 of the IS900 PCR negative suspensions was evaluated, using a PCR/crossblot technique. In 1 out of 32 samples a positive result was obtained with a IS900 based *M. paratuberculosis* specific probe, indicating a false-negative result in the IS900 PCR. In all other suspensions the strongest reaction patterns were observed with either a probe against the *M. avium*-complex (in the absence of a reaction with a IS900 specific probe) or with a probe against *M. phlei*. However, in all crossblot patterns also other reactions were observed against other mycobacterial species, like *M. intracellularis*, *M. gordii*, and *M. smegmatis*, indicating the heterogeneous nature of these crude suspensions. In our opinion, these results once again underline, that Ziehl-Neelsen positive cultures should be positively identified as *M. paratuberculosis*, either by demonstrating the mycobactin dependency or by a specific IS900 PCR.

Title Transposon mutagenesis in *Mycobacterium paratuberculosis* using the shuttle phasmid ph AE94.

Author(s) Cavaignac SM, White SJ, de Lisle GW, Collins DM.

Institution AgResearch, Wallaceville Animal Research Centre, Upper Hutt, New Zealand.

Abstract Transposon mutagenesis has been used extensively in prokaryotes to produce random libraries of chromosomal mutants which have been used to investigate the genetic determinants responsible for particular phenotypes. The ability of this approach to provide direct identification of the mutation site makes it an extremely powerful technique for analysing genetic function. In this study, insertion mutants of *Mycobacterium paratuberculosis* were made by using the conditionally-replicating shuttle phasmid ph AE94. This phasmid consists of an *Escherichia coli* plasmid containing Tn5367 which replaces an inessential region of a temperature-sensitive variant of mycobacteriophage TM4. Two libraries each containing

thousands of independent mutants were made in the New Zealand cattle strain M. paratuberculosis 989 and in the reference strain M. paratuberculosis TMC1613. Southern blotting of 20 kanamycin-resistant mutants using Tn5367 as a probe indicated that that random transposition had occurred. Transposon mutants of M. paratuberculosis were screened for auxotrophy, carbon source preference and altered cell morphology. DNA has been isolated from mutants identified in the screening process and the region surrounding the transposon insertion has been isolated and cloned. Primers homologous to regions near the ends of the transposon have been used to sequence out from the transposon to determine the chromosomal sequence out from the transposon to determine the chromosomal sequence at the insertion site.

Title Sequences of Multi-PCR Products in Variants of Mycobacterium avium subsp. paratuberculosis.

Author(s) Nishimori K, Uchida I, Eguchi M¹, Tanaka K, Tachibana S², Nakaoka Y³, Nishimori T, Imai K.

Institution Hokkaido Research Station, National Institute of Animal Health, Hitsujigaoka-4, Toyohira, Sapporo, 062-0045, ¹ National Institute of Animal Health, Kannondai-3-1-1, Tsukuba, 305-0856, ² Ishikari Livestock Hygiene Service Center, Hitsujigaoka-3, Toyohira, Sapporo, 062-0045, and ³ Tokachi Livestock Hygiene Service Center, Kisen 59-6, Kawanishi, Obihiro, 089-11, Japan.

Abstract Six isolates of Mycobacterium avium subsp. paratuberculosis from 1992 to 1995 possessed multi-PCR products (229bp and the higher molecular products), using primer 1 (139-162) and primer 2 (313-367), common IS900-detecting primer pairs. The sequences of the higher molecular products revealed that there was the 55bp-direct repeat from 139 to 193 of the IS900 element upstream of it. The PCR profile of the variants was the mixture of both the profile of the template DNA from the cloning vector with the 229bp-band and that with the higher band. It was speculated that there were 2 kinds of insertion of IS900 in the variants.

Title An error in the reported IS900 nucleotide sequence affects the proposed expression of ORF2 (hed) and the detection of M. paratuberculosis using the polymerase chain reaction.

Author(s) Willemsen PTJ, Bakker D, van Zijderveld FG.

Institution Institute for Animal Science and Health, Lelystad, The Netherlands.

Abstract Sequence analysis of IS900 of several M. paratuberculosis strains from different origin revealed the presence of an additional 'GC' pair at position 36, when compared with the published nucleotide sequence data. As a consequence, transcription of the IS900 element into mRNA results into a putative open reading frame ORF2 or hed, with a termination codon (UAA) just prior to the 3' end of the insertion element. The transcriptional organisation is therefore identical to homologous insertion elements like IS901/902 and IS116 of M. avium and S. clavuligerus respectively. In other words: using the promoter sequence provided at the insertion site by the host for the expression of ORF2 and providing itself a ribosomal binding site to the open reading frame present in the interrupted operon at the insertion site. Therefore, insertion of the IS900-element is not likely to disrupt the expression of genes present at this site. In addition, detection of M. paratuberculosis by PCR using primers based on the published sequence including this error (eg. primer p90) will be severely hampered, by affecting both the specificity and sensibility of the reaction as shown by quantitative PCR based on Taqman technology.

Title Isolation and Sequencing of Insertion Element IS1311 from Mycobacterium paratuberculosis.

Author(s) Lambeth M¹, Griffin F¹, Crawford A², Mackintosh C³.

Institution ¹ Disease Research Lab., Department of Microbiology, University of Otago. ² AgResearch Molecular Biology Unit, Department of Biochemistry, University of

Otago.³ AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand.

Abstract The mycobacterial family of insertion elements include the species specific elements IS61101 and IS9002, which are used as identification tools for *M.tuberculosis* complex and *M. paratuberculosis* respectively. The *M. avium* elements of this family, IS1311 and IS1245, have been used for strain differentiation³. Collins et al⁴ have recently described the presence of IS1311 in *M. paratuberculosis* through Southern hybridisation. Here we describe a screening strategy for a *M. paratuberculosis* genomic library resulting in the isolation of a clone bearing IS1311. Sequencing of this clone has revealed an insertion element with very high homology to the *M. avium* sequence.

Title Detection of Mycobacterium paratuberculosis in faecal samples by polymerase chain reaction: Neutralisation of faecal inhibitory substances.

Author(s) Mori Y, Oki M, Muneta Y, Shimoji Y, Yokomizo Y.

Institution National Institute of Animal Health, Tsukuba, Japan.

Abstract Polymerase chain reaction (PCR) amplification of IS900 gene is recognised as a rapid method for the direct detection of Mycobacterium paratuberculosis from faecal samples. However, because of the problems associated with inhibitory activity of faeces, organisms or DNA templates have to be purified from faeces. These sample preparation steps are laborious and time consuming to perform PCR with large number of samples. Using the faecal samples taken from experimentally infected calves and artificially seeded faecal samples, several methods were evaluated for their ability to reduce PCR inhibitory activity of faeces. A nested PCR reported by Collins et al. was employed for the amplification of IS900 gene fragment, and the amplified products were electrophoresed on 2.5% agarose gels and were stained ethidium bromide. It was found that this inhibitory activity of faeces could be reduced by addition of Ampdirect((Shimadzu, Japan) that neutralise PCR amplification inhibitors in blood and faeces. Furthermore, PCR amplifiable DNA could be prepared by using InstaGene(Matrix (Bio-Rad, USA) with simple procedures. In testing artificially seeded faecal samples, PCR product was detected at a concentration of 1.25% to 0.3% of faeces by addition of Ampdirect, whereas it had to be diluted 8 to 16 times more for PCR without Ampdirect to get positive reaction. Some of faecal samples from experimentally infected calves that were negative in PCR without Ampdirect showed strongly positive reaction with PCR using Ampdirect. Thus, the combination of DNA extraction with InstaGene Matrix and Ampdirect-PCR might be a rapid and simple method for the direct detection of *M. paratuberculosis* in faeces.

Title Comparison between PCR and classical tests applied to paratuberculosis diagnosis in different animal species in a farm.

Author(s) Caracappa S¹, Lillini E², Reale S¹, Vitale F¹, Borghese A³, Fagiolo A².

Institution ¹ Istituto Zooprofilattico Sperimentale della Sicilia, Palermo (Italy). ² Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Roma (Italy). ³ Istituto Sperimentale per la Zootecnia, Roma (Italy)

Abstract PCR technique was performed on faecal material and tissue samples taken post-mortem from the same animals in order to make paratuberculosis diagnosis in different ruminant species. The samples were taken from an herd in a countryside around Rome. We investigated serologically positive cattle, goats and water-buffaloes with specific symptoms of Johne's disease. Other animals with not specific signs of the pathology were analysed. Biomolecular tests were compared with classical serological and microbiological methods. For PCR test, specific oligonucleotide primers were chosen inner to IS 900 sequence to amplify a 400-bp fragment. To confirm the results a 229-bp was designated by nested PCR from the first amplification product. Sensitivity and specificity of the methods were established. The infection can spread in environment and pass through different animal species living in the same farm. Biomolecular technique may be utilised as tool for validation of paratuberculosis diagnosis with different

methods and for detection of latent forms of disease. It may be useful to avoid the spreading of infection in the farms and in the environment.

Title Improved reliability of diagnostic PCR with an internal positive control molecule.

Author(s) Englund S, Ballagi-Pordány A, Bölske G, Johansson KE.

Institution National Veterinary Institute, Uppsala, Sweden

Abstract To check the efficiency and the sensitivity of the polymerase chain reaction, it is advisable to apply standard molecules as indicators of the procedures. It is important to detect false negative results depending on pipetting errors or inhibition of the amplification, often observed when PCR is performed with complex samples, such as faeces and tissues. We have developed internal control molecules, termed mimics, to be used in two diagnostic PCR systems for the detection of the *M. avium* subsp. paratuberculosis specific element IS900. Two oligonucleotides with the IS900 PCR primers p36 and p11 as 5'overhang and two oligonucleotides with the IS900 nested PCR primers s204/s347 and s749/s535 as 5'overhang, were constructed to amplify a region of 387 bp and 635 bp, respectively, of a segment of the human actin gene inserted into the pUC18 plasmid. The resulting mimics had the target sequences for the IS900 PCR primers at both ends. The mimic amplicons were cloned in PCR-Amp SK(+) cloning vector and the purified plasmid was used in the PCR. The amount of mimic that could be used in the PCR without affecting the amplification of the segment of IS900, was determined to be 103-104 molecules in single PCR and 10 molecules in nested PCR. The use of mimics in PCR was evaluated with spiked samples and clinical samples which were previously found positive in culture. When *M. avium* subsp. paratuberculosis DNA was present at high concentration in a sample, the IS900 PCR product was detected, but not the mimic, due to competition. We did not observe any competitive effects on the IS900 amplification when the mimic was included in the PCR. The identical primer-binding nucleotide sequences allowed co-amplification of the IS900 element and the mimic in the same tube, and simultaneously, the size difference allowed easy discrimination between their PCR products. Using the mimic undoubtedly facilitated the interpretation of negative PCR results and it was easy to identify samples which were inhibiting the amplification.

Title Use of a PCR Method on Faecal Samples for Diagnosis of Sheep Paratuberculosis

Author(s) Garrido JM, Cortabarria N, Aduriz G, Juste RA.

Institution Neiker (Instituto Vasco de Investigación y Desarrollo Agrario) (SIMA(. Berreaga, 1. 48160 Derio, Bizkaia.

Abstract The high sensitivity of PCR compared to the difficulties of faecal culture in sheep prompted the development of PCR protocols for detection of *M. a. paratuberculosis* DNA in sheep faeces. Although the PCR itself is well developed, and does not pose big technical problems, concentrating the bacteria from samples that may contain low numbers of bacilli using practical methods is still the main difficulty. In this study we compare several protocols for concentration and purification of *M. a. paratuberculosis* DNA from faecal samples. In a preliminary phase we used a single faecal sample of a natural case of sheep paratuberculosis to define the conditions for best relative sensitivity. In a second phase, we used a freezing-thawing protocol relatively easy to scale up to examine faecal samples of a group of selected sheep from different flocks of known individual serological, pathological and cultural paratuberculosis status. On this sample, the PCR protocol showed a sensitivity of 71.5%, and an specificity of 92.3%.

Title Use of immunomagnetic PCR (IMS-PCR) to aid the diagnosis of Johne's disease.

Author(s) Mason O¹, Grant IR¹, Ball HJ², Rowe MT¹.

Institution ¹ Department of Food Science (Microbiology), The Queen's University of Belfast, and ² Veterinary Sciences Division, Department of Agriculture for N. Ireland, Belfast, N.

Ireland, UK

Abstract Molecular methods have the potential to be a valuable tool in the diagnosis of Johne's disease (JD). However, the direct application of IS900 PCR to faeces is hindered by the presence of constituents in the faecal material inhibitory to the Taq DNA polymerase enzyme. An immunomagnetic PCR (IMS-PCR) technique, recently developed at Queen's University to facilitate the selective isolation of *M. paratuberculosis* from milk, was adapted for application to faeces. Faeces samples were diluted (1:5) in PBS-0.05% Tween 20, shaken vigorously by hand and then centrifuged (300 x g for 3 min) to quickly sediment particulate matter. One ml of the resultant supernatant was subjected to immunomagnetic separation (IMS). Positive PCR signals were obtained after IMS from faeces spiked with as few as 10 CFU of *M. paratuberculosis* per g. In contrast, when IMS-PCR was applied to stomached faeces samples, a PCR signal was only obtained when high numbers of *M. paratuberculosis* were present, indicating that low-speed centrifugation more effectively removed particulate matter. A comparative blind trial was carried out on 95 faeces samples, chiefly of bovine origin. Each faeces sample was subjected to microscopic examination after Ziehl-Neelsen (ZN) staining, IMS-PCR, and HPC decontamination and culture. Results showed that IMS-PCR detected more infected samples than ZN smear, and as many infected samples as culture. This IMS-PCR assay could aid the more rapid diagnosis of JD.

Title Detection of *Mycobacterium avium* subspecies paratuberculosis in free-ranging bison by PCR.

Author(s) Ellingson JLE¹, Stabel JR¹, Whitlock RH².

Institution ¹ USDA/ARS/National Animal Disease Center, Ames, IA, USA and ² University of Pennsylvania, New Bolton Center, PA, USA.

Abstract Bacteriologic culture is considered the "gold standard" method for diagnosing paratuberculosis infection. However, bacteriologic culture of MAs paratuberculosis is time consuming and laborious and the success of bacteriologic culture varies with the species of animals tested. Improved diagnostic tests are needed that can be used in domestic and wild ruminants. PCR has been used for the detection of MAs paratuberculosis DNA in faeces and tissues. However, elaborate specimen preparation protocols are used and may result in reduced sensitivity of these assays. We applied the PCR technique to detection of paratuberculosis in twenty-five free-ranging bison. We report the performance of preassembled PCR mixtures to detect MAs paratuberculosis DNA in frozen ileum, jejunum, and mesenteric lymph nodes from bison. Specific oligonucleotide primers used in the PCR assay were derived from the 16S rRNA (MAs) sequence and an insertion element IS900 (MAs paratuberculosis). Crude genomic DNA samples from tissues were prepared using a simple boiling technique and the samples were tested by PCR. An animal was considered positive if MAs paratuberculosis DNA was detected by PCR amplification in at least two frozen tissues from an animal using the IS900 primer set or by detection of DNA in a single tissue from an animal with both the 16S rRNA and IS900 primer sets. Using these criteria, 15 of 25 (60%) bison tested were positive for MAs paratuberculosis DNA. The data indicate that these free-ranging bison had been exposed to MAs paratuberculosis.

Title Paratuberculosis in Bison: A comparison of PCR, culture and Histopathology.

Author(s) Whitlock RH, West S, Layton B, Ellingson J, Stabel J.

Institution University of Pennsylvania, New Bolton Center, Kennett Square, PA, USDA/ARS/NADC Ames, Iowa.

Abstract Multiple tissues, blood and faecal samples were collected from twenty-four adult Bison, all with clinical signs suggestive of Johne's Disease including chronic weight loss. Tissues were harvested in a way to minimise contamination between tissues taken from the same animal. AGID and ELISA tests were done on the serum samples. No sample was positive on the AGID test while two (#3 & #18) were positive on the CSL/IDEXX ELISA test. The tissues were

examined histopathologically using the H E and Ziehl-Neelsen stain. PCR using the IS-900 sequence was detected *M. paratuberculosis* in 19 of 24 faecal samples and tissues from 21 of 24 Bison were PCR positive where one or more tissues were tested. Tissues from the other three Bison were classified as PCR suspicious, since a faint band was present in the gel. Tissues were culture positive from 15 of the 24 Bison tested while one faecal sample was culture positive while the tissues from that Bison was culture negative. The colonies isolated often grew better on HEYM without pyruvate than on HEYM with pyruvate (4.1 gm/l). Several cultures were first recorded as positive well beyond the typical 14 weeks of culture. Based on experience with Johne's infected tissues of cattle the specific strain of *M. paratuberculosis* seemed much more difficult to isolate from Bison tissues than cattle tissues. Histopathologic examination of the same tissues as were examined by culture and PCR identified 18 animals positive as determined by finding giant cells with acid fast rods with morphology compatible with *M. paratuberculosis*. The histological negative animals were also culture negative but PCR positive. Immunohistologic staining with specific polyclonal antibody stains are pending. Using these criteria, 15 of 25 (60%) bison tested were positive for MAs paratuberculosis DNA. The data suggest that these free-ranging bison had been exposed to MAs paratuberculosis.

Title Ovine paratuberculosis- an emerging disease in South Africa.

Author(s) Michel AL, Bastianello SS.

Institution Onderstepoort Veterinary Institute, South Africa Koen P; Western Cape Veterinary Services, Stellenbosch, South Africa.

Abstract The first case of ovine paratuberculosis in South Africa was diagnosed in an imported Merino ram in 1967. During the late 1980ies outbreaks occurred on two agricultural research farms in Gauteng and Mpumalanga Provinces, respectively. It was not before 1993 that paratuberculosis was confirmed in the Western Cape Province. Following a considerable increase in the number of infected farms it was decided to carry out a nationwide survey using the AGID test. The survey was completed between July 1996 and June 1997. All positive reactors were slaughtered and examined by histopathology. A total of 145,934 samples from 2019 farms were tested. Fifty-four infected farms were identified in the Western and Eastern Cape provinces. Links between infected farms in the two provinces could be established. Examination of the distribution of infected farms in the Western Cape indicated a positive correlation between acid soils and infection. In an attempt to increase the sensitivity and facilitate screening of large numbers of sera in a future monitoring programme two commercial ELISA systems are presently compared. Sera from histologically positive sheep which were slaughtered during the initial disease outbreaks on the two research farms as well as during the survey are used.

Title Paratuberculosis in Goats: Comparison between Serological Diagnosis by AGID and ELISA tests.

Author(s) Vélez-Hernández M¹, Chávez-Gris G¹, Collins MT², Suárez Güemes F¹.

Institution ¹ Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autonoma de Mexico. Circuito Exterior s/no., Ciudad Universitaria. Coyoacan 04510, Mexico City. ² Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison.

Abstract In order to evaluate the behaviour of the AGID and the ELISA tests, for the diagnosis of caprine paratuberculosis, 5 serum sampling were performed on 3 naturally infected caprine herds. In both serological tests the PPA-3 antigen was employed. The AGID test detected goats with and without clinical signs. Beside, the ELISA seemed to require lower concentration of antibodies to trigger a positive result. Significant differences ($P < 0.01$) were observed in the prevalences estimated by the AGID and ELISA tests along the 5 serum sampling. The AGID test shows as an useful serological test able to detect clinical such as subclinical Map infected goats. The behaviour of both tests were mostly the same and seemed not to be affected by the different paratuberculosis prevalences estimated on each flock.

Title Paratuberculosis and Avian Tuberculosis in Red Deer in New Zealand: Clinical syndrome and diagnostic tests.

Author(s) Mackintosh CG¹, Reichel MP², Griffin JFT³, de Lisle GW⁴.

Institution ¹ AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand; ² Central Animal Health Laboratory, Wallaceville, Upper Hutt, New Zealand; ³ University of Otago, Dunedin, New Zealand; ⁴ AgResearch Wallaceville, Upper Hutt, New Zealand.

Abstract Paratuberculosis and to a lesser extent avian tuberculosis, are emerging as potentially serious problems on deer farms in New Zealand. To date, the majority of reported cases have been subclinically affected animals detected at slaughter, usually with mesenteric lymph nodes lesions which are a nuisance value to the venison industry and financial loss to the farmers due to their gross and histological similarity to lesions caused by *Mycobacterium bovis*. Recently however, there have been a number of outbreaks of severe clinical disease in 8 to 15 month old red deer. Serological tests are useful in clinically affected animals although they cannot differentiate between *M. paratuberculosis* and *M. avium*. Studies were undertaken to compare the sensitivity of the agar gel diffusion test, various ELISA tests, the complement fixation test, the lymphocyte transformation test, the comparative skin test and faecal culture for the detection of subclinically infected hinds in a herd of red deer which had experienced a severe outbreak of clinical paratuberculosis in yearling animals. The results will be presented at the conference.

Title Johne's disease diagnosis for non-domestic hoofstock

Author(s) Manning EJB, Collins MT.

Institution School of Veterinary Medicine, Madison, WI. USA.

Abstract Concern about *M. paratuberculosis* infection of non-domestic hoofstock in US zoos is increasing. The Johne's Testing Center has completed radiometric culture assays for up to a third of the 176 zoos accredited in the US. *M. paratuberculosis* has been isolated from samples submitted by 9.1% of these institutions. Three of these institutions have had a significant prevalence of the disease in multiple species. Molecular fingerprinting of isolates is underway to assess the ability of these strain(s) to infect many types of ruminants. Over the last two years, approximately 50% of the mycobacteria isolated from faecal samples have been non-pathogenic, primarily saprophytic, organisms. The remaining 50% have been determined by genetic probe to be *M. paratuberculosis* (38.3%) and *M. avium* (11.7%). For one institution, histopathological confirmation of infection could be made in only 22% of animals for whom the organism was isolated from ante-mortem faecal samples. In an attempt to better characterise these cases, and to develop a more rapid and less expensive means of surveillance in these populations, the Johne's Testing Center has begun an assessment of the usefulness of a protein G ELISA (IDEXX) as a multi-species diagnostic tool. Sera from non-domestic hoofstock (both from confirmed infected animals and from animals belonging to the same species at institutions with no history of Johne's disease) are being tested. To date the binding patterns across numerous species at multiple serum dilutions appear comparable, although the optical density/dilution curves shift up or down depending on the species in question. Results using purified immunoglobulins, statistical analysis of the differences among and between individuals within the same genus/species and across genus/species and a comparison of the ELISA with AGID will be made.

Title Modification of a bovine ELISA to detect camelid antibodies to *Mycobacterium paratuberculosis*.

Author(s) Kramsky JA¹, Miller DS², Hope A³, Collins MT¹.

Institution ¹ Dept. of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, Madison, WI, ² Fort Wayne Children's Zoo, Fort Wayne, IN, ³ Victorian Institute of Animal Science, Attwood, Australia

Abstract Mycobacterium paratuberculosis infection, or Johne's disease, has a reportedly low prevalence in South American camelid populations. Recently, however, single cases in the United States as well as an outbreak of the disease in Australian alpacas have been described. To provide a rapid and cost-effective method of diagnosing Johne's disease in this species, the bovine PARACHEK® Johne's Absorbed EIA (CSL Limited, Victoria, Australia) was modified to create a camelid-specific serum antibody assay. The anti-bovine immunoglobulin was replaced by an anti-llama IgG conjugated to horseradish peroxidase. Using serum from 21 culture-negative and 10 culture-positive camelids, checkerboard titration of principal reagents was performed. Dilutions of key components providing clear discrimination between positive and negative controls were determined. Completion of a kinetic assay determined the optimum optical density at which the enzyme-substrate reaction should be stopped. A separate herd of 100 culture-negative camelids was tested to establish cut-off values. Results were expressed as a percentage of the controls by transforming optical density values to ELISA values (EV%). A preliminary EV cut-off of 20% was established. Using this prototype assay, culture-positive animals showed significantly different antibody responses from culture-negative animals. These results indicate that this camelid-specific ELISA, once refined, may be a useful tool for both diagnosing and screening camelid herds for *M. paratuberculosis* infection.

Title Use of different tests in diagnostics of Mycobacterium paratuberculosis in infected cattle

Author(s) Cvetnic Z¹, Ocepek M², Kovacic H¹, Briek K³, Trstenjak J⁴.

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Abstract During a regular annual tuberculinisation on the farm of dairy cows of Frisian breed 12.1% of unspecific reaction to tuberculin was established. Some of the cows had chronic watery diarrhoea and were markedly skinny, thus such clinical symptoms indicated paratuberculosis. The blood samples of 205 cattle on the farm were serologically analysed using the following methods: enzyme - linked immunosorbent assay - ELISA (IDEXX), complement fixation (CF) test and agar gel immunodiffusion - AGID test (Bioveta), Mycobacterium paratuberculosis gamma interferon test kit-gamma-IFN (IDEXX). The allergy test (P.P.D.-Johnin) was also carried out as well as the DNA - test (IDEXX) from the dung of cattle reacting positively or suspiciously with one of the tests mentioned. Applying some of the tests mentioned above, positive reactions were established in 39(19%) out of 205 cattle examined. When using ELISA test positive reactions were established in 20(9.7%) of cattle, with CF test 3 (1.5%) cows were positive and 9 (4.4%) of them suspected, with AGID test 4 (1.95%) of them were positive and with gamma-IFN test reaction were established in 23 (11.3%) cattle. When using the allergy test in 22 (10.7%) cattle the swelling of skin wrinkler greater than 2mm was established and by DNA - probe positive reaction was established in 10 (25.6%) cattle out of 39 samples analysed. Combining several tests (ELISA, gamma-IFN, DNA-probe) and using faecal culture reliable diagnostics of paratuberculosis in cattle can be obtained.

Title Impact of contaminated environment, low vertebrates and invertebrates by Mycobacterium avium subsp. paratuberculosis on the spread of the disease in ruminant herds with different prevalences.

Author(s) Pavlík I, Horvathova A, Fischer O, Bartl J, Dvorska L, Matlova L, du Maine R.

Institution Veterinary Research Institute, Brno, Czech Republic.

Abstract An eradication programme against paratuberculosis implemented in this institute over ten years has shown that this infection is spread particularly among young animals. To gain a better understanding of the pathogenesis of this diseases, we have started to investigate the effect of the environment (barns and pasture), low vertebrates and invertebrates (worms, diptera and beetles) on disease transmission. From 1993-1998, fourteen ruminant herds (cattle, wild goats and moufflons) were examined. Samples included: 725 from the environment, 34 organs from low vertebrates and 1700 from invertebrates (38 worms, 17 beetles and 1645 diptera). Strains of *M.avium*. subsp. paratuberculosis were isolated from environmental samples originating from infected herds with a prevalence of clinical paratuberculosis >3%. Isolated strains were always of the same DNA type. However, one cattle herd was infected with strains of various DNA types: 55.8% type A-C10, 21.1% type B-C1, 15.4% type B-C9, 5.8% type B-C10 and 1.9% type B-C14. The environment in this cattle herd was contaminated by the most common DNA types: 69.2% type A-C10, 7.7% type B-C1 and 23.1% type B-C9. 17 mice were trapped from a pastoral area, from which two strains of *M.a.paratuberculosis* were isolated. However, we were unable to determine their DNA fingerprint type. In addition, identification of *M.a.paratuberculosis* in samples collected from invertebrates was unsuccessful. Dungworms, flies (at different stages of life-cycle) and beetles were either artificially infected with *M.a.paratuberculosis* from intestines of sick cows or with faeces from cattle and goats. After 4-6 weeks, *M.a.paratuberculosis* could be isolated from these faeces. In addition, *M.paratuberculosis* (with the same DNA type) could be detected for up to 12 months after the infection in faeces of more than 300 hamsters, whilst in liver and spleen, survival was even longer (24 months). Our research was partially supported by the Ministry of Agriculture (grant no. EP0960006087) and the Ministry of Health of the Czech Republic (grant no. 4211-3/97). Permanent address of Robin du Maine - Hogeschool van Utrecht, Netherlands.

Title Pathology observed in natural cases of paratuberculosis in goats.

Author(s) Pérez V, Corpa JM, García Marín JF.

Institution Departamento de Patología Animal: Medicina Animal (Anatomía Patológica). Facultad de Veterinaria. Universidad de León. Campus de Vegazana, s/n. 24071 León (Spain).

Abstract A description and classification of lesions associated to natural paratuberculosis infection has been made in goats, culled for any reason, from flocks with high number of clinical cases. A total of 136 adult goats, with and without clinical signs, have been evaluated by pathological methods. Samples from the ileocaecal valve, ileum, jejunum and mesenteric and ileocaecal lymph nodes were examined. Different types of lesion could be observed. The most remarkable findings were the following: - The presence of focal granulomatous lesions, tuberculoid type, associated exclusively to the organised intestinal lymphoid tissue (Peyer's patches), mainly located in the ileocaecal valve. They were consistent with similar lesions previously described in sheep. - The description of lesions consisting only in the presence of granulomata formed by macrophages, located exclusively in the apex of the intestinal villi, without causing the thickening of the wall and associated with severe clinical signs. - The observation of severe lesions characterised by an intense necrosis and calcification of the lymph nodes and the lymph channels of the serosa and the mesentery, in a high number of goats with severe diffuse lesions.

Title Evaluation of the sensitivity and specificity of a commercially available absorbed ELISA for the detection of antibodies against *Mycobacterium paratuberculosis* in cattle and performance of this ELISA in a herd certification program.

Author(s) Van Maanen C, Swinkels M, Kalis CHJ, Barkema HW, van Weering HJ.

Institution Animal Health Service, The Netherlands.

Abstract At the Animal Health Service in the Netherlands initially an absorbed ELISA (IDEXX/CSL) based on the use of an anti-bovine conjugate was validated. For the European market, the manufacturer replaced this test by an absorbed ELISA with a protein G conjugate (HerdCheck M. pt., IDEXX, Scandinavia). We evaluated the specificity and relative sensitivity - as compared with faecal shedding - of this new commercially available absorbed ELISA (test B),

in comparison with the previously validated IDEXX/CSL ELISA (test A). For estimation of the sensitivity, positive faecal culture was taken as a gold standard. Serum samples were obtained from 74 faecal culture positive cows. For test A and B, we found a sensitivity of 35 and 49 %, respectively. On a panel of 199 sera from once or twice faecal culture positive cows, belonging to low prevalence herds participating in an eradication program, we found a sensitivity of 27,3 % and 36,9 %, respectively. For test B, at a higher cut-off (S/P ratio > 0.3), for these serum panels the sensitivity was 40% and 27.8 %, respectively. The specificity of test A was evaluated using 821 sera from 36 herds, all herds being tested three times negative by pooled faecal culture. We found a specificity of 99.4 %. The specificity of test B was initially evaluated on a subset of 50 sera from three highly unsuspected Dutch herds and on a set of sera from France (n=260) and the USA (n=255) from herds that were found repeatedly negative after individual faecal culture. We found a specificity of 100 %, 99.4 % and 99.6 %, respectively. However, using a larger panel (n=339) from three highly unsuspected Dutch herds, a specificity was found of 98%. We decided to interpret results of test B at a higher cut-off (S/P ratio > 0.3) corresponding with a specificity of 99.4 % and a relative sensitivity of 40% for our herd certification program. In this voluntary program, all cows of three years and older are tested in the absorbed ELISA, and all serological reactors are confirmed by individual faecal culture. Preliminary results show, that 30 out of 46 ELISA positive (S/P ratio > 0.3) cows were confirmed by faecal culture, and a correlation was observed between S/P ratio and percentage of positive cultures. For a more extensive evaluation of the specificity of test B in Dutch herds, 1377 blood samples were taken from 28 herds, all herds being tested four times negative by pooled faecal culture. Surprisingly, at the relative high cut-off we use (S/P ratio > 0.3) we found a specificity of 96.5 % corresponding with 48 seropositive cows. However, 36/48 positives originated from five herds. Excluding these herds from the data set yielded a specificity of 99%. Further research is carried out to decide whether these seropositives are true-positives or false-positives.

Title Evaluation of the sensitivity, specificity and detectability of a serological ELISA test for the diagnosis of Paratuberculosis.

Author(s) Couquet C, Pourquier P².

Institution ² Institut Pourquier, Montpellier, France.

Abstract This study concerns the relative value of a serological ELISA test compared to the methods of complement fixation test (CFT) and faecal culture habitually used for Paratuberculosis screening. The specificity study of the ELISA technique was conducted on adult animals from herds in the Haute-Vienne recognised as disease-free and having had negative faecal culture results for 5 years (n = 522). It was conducted too by analysis at the Departmental Veterinary Laboratory of the Aveyron of a population of animals less than 24 months old from herds not presenting symptoms but without a known history in terms of faecal culture for Paratuberculosis (n = 1000). The examination of these populations enabled us to observe a specificity for the ELISA technique that was greater than or equal to 99.9. The relative ELISA detectability was evaluated in comparison to CFT by analysis of successive dilutions of strongly positive sera. This study enabled us to ascertain that the evaluated ELISA method was able to provide positive results with antibody concentrations as much as 4 times lower than those yielding positive results with CFT. This observation helps to explain, in particular, the frequency of conflicting CFT negative/ELISA positive results. The relative sensitivity of ELISA and faecal culture was evaluated by analysing, every 90 days for two years, all of the animals in an infected herd (n = 80), of high genetic value, with regular follow-up, evolving in natural conditions. This study enabled us to establish that in two-thirds of the cases, the use of the serological test permitted an earlier diagnosis than with faecal culture. In one third of the cases, the use of the faecal culture technique led to an earlier diagnosis than the ELISA technique. The use of these tests in combination or ELISA alone is debated.

Title Evaluation of a LAM-based ELISA for the detection of subclinically paratuberculosis-infected cattle

Author(s) Dekker C¹, Rehm T², Koets AP³, vom Schloss A, Leuven G⁴, Strutzberg K⁵, Homuth M⁵.

Institution ¹ SVANOVA Biotech, Uppsala, Sweden, ² Department of Microbiology and Infectious Diseases, School of Veterinary Medicine Hannover, Germany, ³ Institute of Infectious Diseases and Immunology, Utrecht University, The Netherlands, ⁴ Staatliches Veterinäruntersuchungsamt Krefeld, Germany, ⁵IVD GmbH, School of Veterinary Medicine Hannover, Germany

Abstract An ELISA, using M. paratuberculosis - lipoarabinomannan (LAM) as antigen was evaluated by comparing serological (several ELISAs, CFT) and cultural methods (culture of faeces and ileocaecal lymphnodes) in herds with and without a paratuberculosis history in several areas of Germany and the Netherlands. The LAM-ELISA showed a high discriminatory efficacy and reproducibility. The positive and negative predictive value in infected herds were 76 resp. 85 % with respect to a gold standard (culture of ileocaecal lymphnode). This ELISA was used in an eradication-program in Northern Germany. In combination with faecal culture and hygienic measures an effective decrease of seroprevalence in the herds could be achieved.

Title Optimisation and Evaluation of an absorbed ELISA (enzyme - linked immunosorbent assay) in identifying Johne's disease (Mycobacterium paratuberculosis) in Alpaca (lama pacos).

Author(s) Ganci DA, Hope AF, Carajias M.

Institution Victorian Institute of Animal Science. Agriculture Victoria. Victoria. Australia.

Abstract A bovine monoclonal and two polyclonal conjugates, anti-llama and anti-alpaca were optimised and evaluated for a potential alpaca absorbed ELISA. The optimum conditions and hence the ability of each assay to reduce non-specific EIA reactions was measured by calculating the positive to negative ratio (P/N). The calculated P/N ratio for the anti-llama and anti-alpaca systems were 4.04 and 3.57 respectively. A P/N ratio of 5.57 was calculated for the bovine monoclonal. Based on its greater P/N ratio the bovine monoclonal ELISA was subsequently evaluated for specificity and sensitivity against five different cut-points aiming to maximise the specificity of the assay. The first two cut points were derived by the addition of 0.1 and 0.2 to the mean optical density of the specificity panel and was based on the CSL absorbed ELISA. The third and fourth cut-points required obtaining ratios by calculating the mean test optical density divided by the mean specificity panel optical density. The final cut-point involved calculating one standard deviation above the mean of the specificity panel. The specificity panel comprised of sera from 320 uninfected healthy alpaca with no history of Johne's disease. These animals were held for some time at Cocos Island quarantine station. The sensitivity panel consisted of sera from 16 alpacas with subclinical and clinical disease. A specificity of 92% and a sensitivity of 31% were obtained when the cut-point of one times the mean standard deviation of the negative sera was selected. The repeatability of the assay was assessed for variation within a plate and variation between days. The mean coefficient of variation among wells was 6.79% and between days was 12.65% for the positive and negative controls.

Title The Australian National Serum Reference Panel for Johne's disease.

Author(s) Hope AF, McDonald WL, Kluver PF, Tulk PA, Waldron BR, Hughes C, Carajias M, Walker SK, Rhode ME.

Institution Victorian Institute Of Animal Science, Attwood, Australia.

Abstract The bovine National Serum Reference Panel (NSRP) established in 1996, is integral to the Quality Plan for Johne's disease testing in Australia. This panel of sera is used to address key areas of quality assurance of Johne's disease enzyme immunoassays (EIA), standardisation of interlaboratory testing and quality control of testing within laboratories. Currently, NSRP contains 518 sera collected Australia-wide and comprised of 241 sera from Victoria, 14 from New South Wales, 3 from Tasmania, 200 from Western Australia, 44 from Northern Territory and 16 from Queensland. These sera have a range of optical densities and originated from infected and uninfected cattle classified by histopathological examination and tissue culture. The Johne's disease status is available for animals which were slaughtered or tissue samples

were submitted for examination. Sera are being characterised by repeated testing in order to define their normal range and to identify sera that alert to variability of performance in biological components of EIA. Whilst reproducibility of EIA can be evaluated using sera from NSRP, changes in sensitivity can not yet be assessed. It is anticipated that the next phase of the Quality Plan will focus on collection of sera for sensitivity testing of EIA, especially for testing new batches of kits released for control programs and national market assurance programs.

Title Sensitivity of diagnosis of ovine Johne's disease in New South Wales sheep flocks with a low prevalence of disease.

Author(s) Fraser CA¹, Marshall DJ¹, Ottaway SJ¹, Reddacliff LA², Whittington RJ².

Institution ¹ NSW Agriculture, Orange Agricultural Institute, Forest Rd, Orange, NSW 2570 Australia. ² NSW Agriculture, Elizabeth Macarthur Agricultural Institute, PMB 8, Camden NSW 2800 Australia

Abstract Serology is currently used as the primary screening test for flock diagnosis of ovine Johne's disease in New South Wales. We have previously shown that in flocks where there is a high prevalence of disease (>6%), detection of infection can be significantly enhanced by biasing the test sample to include sheep of low body weight or condition score. This study reports the association between low bodyweight and condition score and the presence of Johne's disease in flocks where there is a low prevalence (<5%) of disease. We sampled 1,300 sheep from 5 flocks known to be infected with Johne's flocks. These flocks were derived from 4 different properties. All sheep were individually identified by ear tag and their bodyweight and condition score recorded. Serum collected from each sheep was tested with AGID and absorbed ELISA techniques, and a 5cm portion of terminal ileum collected from each sheep at slaughter was processed for histological examination. The prevalence of Johne's disease in each flock was low and no greater than 5% depending on the diagnostic method used. Use of biased sampling techniques based on low bodyweight and condition score did not always enhance detection of infection in the flocks studied. Further work is necessary to develop the best strategy for detection of Johne's disease in flocks where there is likely to be a low prevalence of disease.

Title Importance of sample site in histological diagnosis of Johne's disease in sheep.

Author(s) Marshall DJ¹, Fraser CA², Seaman JT¹, Moloney BJ¹, Bailey GD¹.

Institution NSW Agriculture, ¹ Regional Veterinary Laboratory and ² Orange Agricultural Institute, Orange, 2800, NSW, Australia.

Abstract Histopathology is used as the definitive diagnostic test for ovine Johne's disease in Australian sheep flocks. The cost of this diagnostic method is a major limitation on its more widespread use. These costs can be reduced significantly by decreasing the number of tissues submitted for processing to those vital for diagnosis by this means. The Australian Standard Diagnostic Technique (ASDT) nominates that ileum (3 sites, 1 metre apart, the first being adjacent to the ileocaecal valve), colon, caudal jejunal lymph node and ileocaecal lymph node (1 site each) must be sampled from each sheep submitted for diagnosis of Johne's disease. Tissues from 400 sheep with histological evidence of ovine Johne's disease were studied. These originated from sheep either selected by the owner as displaying clinical Johne's disease and submitted to the Regional Veterinary Laboratory, Orange for necropsy (n=250), or from formalin fixed tissues submitted following field necropsy of animals selected on the basis of positive serology (Agar Gel Immunodiffusion Test) in a surveillance testing program (n=150). The frequency of positive histological lesions and the presence of acid-fast bacilli (AFB) for the tissues nominated by the ASDT within this population are reported. All sheep had diagnostic histological lesions in either the terminal ileum (adjacent to the ileo-caecal valve) or the caudal jejunal lymph node. Examination of additional tissues was not warranted and only increased the cost of histological diagnosis. While widespread sampling may be necessary to detect the very early stages of infection by histological means, the additional sites nominated in the ASDT are unlikely to enhance the sensitivity of histological diagnosis.

Title Reproducibility of a faecal culture method for *Mycobacterium paratuberculosis*.

Author(s) Visser I.

Institution Animal Health Service, PO Box 361 9200 AJ Drachten, The Netherlands.

Abstract Faecal samples (40 g) were collected from 22 dairy cows. The cows were chosen because *Mycobacterium paratuberculosis* was detected in faecal cultures 6 to 8 months earlier. Each faecal sample was distributed over ten split-samples. Each split-sample was decontaminated by mixing 3 g faeces and 8 mL NaOH 4% in a mortar to create a homogeneous suspension. This suspension was put in a centrifuge tube and shaken for 15 min. After centrifugation during 15 min at 2500 rpm the supernatant was removed. To the pellet 5 mL oxalic acid (5%) and 0.1% malachitgreen were added. This mixture was shaken for 15 min, followed by centrifugation (15 min, 2500 rpm). The supernatant was removed and to the pellet 6 mL of an antibiotic mixture of Amphotericin and Neomycin was added, vortexed briefly and incubated for 18-24 h at room temperature (20-22 °C). Then 0.8 mL of the sample was distributed over 4 culture tubes with Lowenstein - Jensen medium. The inoculum was taken just above the settled pellet. The culture tubes were placed in an incubator at 37°C in slanted position for one night during which time some evaporation took place, after this the tubes were placed vertical while the caps were firmly fastened. The media were checked for typical colony forming units (CFU) of *M. paratuberculosis* after an incubation period of 6, 8, 12 and 16 weeks. The number of positive tubes after 6, 8, 12 and 16 weeks was respectively: 82 (9.3%), 236 (26.8%), 293 (33.2%) and 347 (39.8%). There were 13 split-samples with only one tube positive after 16 weeks. The number of positive split-samples after 6, 8, 12 and 16 weeks was respectively: 22 (10%), 87 (39.5%), 106 (48.2%) and 110 (50%). The distribution of the positive split-samples varied between 0 to 10 per cow-sample. After 16 weeks 7 cow-samples were negative in all ten of the split samples, 3 cow-samples resulted in only one positive split-sample, 1 cow-sample had three positive split-samples, 1 cow-sample had eight positive split-samples, 4 cow-samples had nine positive split samples and 6 cow-samples were positive in all ten split-samples. In two samples of these six heavy shedders all tubes were positive as early as 6 weeks incubation with numbers between 10 to 100 CFU per tube. It is concluded that *M. paratuberculosis* is not homogeneously distributed in faeces, but present in small clusters. Therefore from cows with low numbers of *M. paratuberculosis* in the faeces, faecal cultures may not necessarily give positive results.

Title Specific Seroreactivity of Crohn's Disease Patients Against P35 and P36 Antigens of *M. avium* subsp. paratuberculosis.

Author(s) Naser SA, Hulten K, Shafran I, Graham DY, El-Zaatari FAK.

Institution Baylor College of Medicine and VAMC, Houston, Texas, and University of Central Florida, Orlando, Florida, USA.

Abstract Crohn's disease (CD) is a chronic inflammatory bowel disease that is similar to Johne's disease in ruminants. Recent data have strengthened the association of *M. avium* subsp. paratuberculosis (*M. para*) with Crohn's disease. To provide more evidence of an etiological association, antibody reactivities of CD patients were tested by immunoblotting against recombinant antigens that were identified previously from our *M. para* genomic library. Two clones [designated pMptb #40 (3.2-kb insert) and #48 (1.4-kb insert)] expressing a 35K (p35) and 36K (p36)-antigens showed specific reactivities with serum from CD patients. The summary of results is as follows: Antigens Positive samples/Total tested (% +ve) PPV NPV
 CD Controls Ulcerative colitis % p36 alone 79/89 (89) 7/50 (14) 10/29 (34) 9389 p35 alone 40/53 (75) 5/35 (14) 1/10 (10) 9378 p35 & p36 39/53 (74) 0/35 (0.0) 1/10 (10) 9876
 PPV = Positive predictive value (specificity); NPV = Negative predictive value (sensitivity) A statistical significance ($p < 0.001$) is observed when the results from CD serum samples reacting with either or both antigens are compared to the controls. The reactivity of anti-*M. para* (specifically against p35 and p36 antigens) antibodies in a significant proportion of CD patients would suggest a causal role for the organism in both diseases.

Title Detection of *M. avium* subsp. paratuberculosis spheroplasts in paraffin embedded tissues by in situ hybridisation.

Author(s) Hulten K, Karttunen TJ, Naser SA, Graham DY, El-Zaatari FAK.

Institution Baylor College of Medicine and VAMC, Houston, TX, University of Central Florida, Orlando, Florida, USA, and University of Oulu, Oulu, Finland.

Abstract Spheroplast form of *M. avium* subsp. paratuberculosis (*M. para*) has been implicated in the pathogenesis of Crohn's disease (CD) and sarcoidosis. Its detection deep in patient tissues would support its association with human disease. However, distinction between the acid-fast and the cultured spheroplast forms in tissue of CD and sarcoidosis patients infected with *M. para* is difficult. To help identify spheroplast forms in inflamed tissues of patients, a non-radioactive in situ hybridisation method was developed and optimised. We have shown that by in situ hybridisation with the IS900-specific probe we can detect nucleic acids from *M. para* spheroplasts, but not their acid-fast forms in tissue sections. Our in situ hybridisation assay is proven specific by the negative finding with control tissue preparations containing spheroplasts of related Mycobacteria (*M. tuberculosis* and *M. smegmatis*) and unrelated organisms (*Helicobacter pylori* or *E. coli*). This novel in situ hybridisation procedure will for the first time provide a way to distinguish between acid-fast and spheroplast forms of *M. para* and to localise them in tissues. Detection of *M. para* variants deep in patient's tissue would be an enormous step forward in the search for an etiological agent in CD.

Title Detection of Mycobacterium paratuberculosis in milk by immunomagnetic PCR (IMS-PCR).

Author(s) Grant IR¹, Pope CM¹, O'Riordan LM¹, Ball HJ², Rowe MT¹.

Institution ¹ Department of Food Science (Microbiology), The Queen's University of Belfast, and ² Veterinary Sciences Division, Department of Agriculture for N. Ireland, Belfast, N. Ireland, UK.

Abstract An immunomagnetic separation (IMS) technique was developed to facilitate the selective recovery of Mycobacterium paratuberculosis from milk. Purified rabbit polyclonal antibody (IgG) raised against radiation-killed intact *M. paratuberculosis* cells was used to coat Sheep

anti-rabbit IgG Dynabeads®. Trials showed that IMS selectively recovered *M. paratuberculosis* from milk spiked with 10-100 CFU of *M. paratuberculosis* when 10 µl of coated beads (10^6 beads) were added to 1 ml of milk and incubated for 30 min at RT. During IMS components of the milk inhibitory to the PCR reaction are effectively removed and therefore IS900 PCR can be successfully applied to milk. Template DNA for IS900 PCR was obtained by heating the bead-cell suspension in a thermal cycler at 100°C for 15 min. Centrifugation ($2,500 \times g$ for 20 min) was employed to concentrate larger volumes of milk (10 and 50 ml) prior to IMS in order to increase the sensitivity of the IMS-PCR assay. IMS-PCR was capable of detecting 10^3 CFU of *M. paratuberculosis* per 50 ml of milk (equivalent to 20 CFU/ml). A blind trial (40 milk samples), consisting of spiked and unspiked, raw and laboratory pasteurised milk samples, showed that IMS-PCR correctly identified spiked milk samples before and after laboratory-pasteurisation. Further studies confirmed that IMS-PCR was able to detect natural *M. paratuberculosis* infection in a raw goat milk sample and several raw sheep milk samples from a herd with a history of Johne's disease. The development and evaluation of this novel IMS-PCR technique will be described.

Title Detection and enumeration of *Mycobacterium paratuberculosis* in milk

Author(s) Herman L¹, Reybroeck W¹, D'Haese E², Nelis HJ².

Institution ¹ CLO-DVK, Melle, Belgium; ² RUG, Lab. Farmac. Microbiol., Gent, Belgium.

Abstract A PCR-detection method for *M. paratuberculosis* in milk has been evaluated. Raw milk, aseptically drawn, was spiked with decimal dilutions of *M. paratuberculosis* cells. Enumeration was performed by: epifluorescence microscopy (DEFT), solid phase cytometry (ChemScan; Chemunex) after viability labelling with a fluorogenic substrate for esterase and by BactoScan 8000 (Foss Electric). For PCR detection, the milk was first chemically or enzymatically destroyed and the bacterial cells were collected by centrifugation according to Herman et al. (Appl. Environ. Microbiol. 61:817-819, 1995) and Rijpens et al. (Appl. Environ. Microbiol., 62:1683-1688, 1996), respectively. *M. paratuberculosis* was specifically detected by a nested PCR according to Millar et al. (Appl. Environ. Microbiol. 62:3446-3452, 1996). The enzymatic destruction of the milk resulted in a 10 times higher detection sensitivity compared to the chemical destruction. This is probably due to the affinity of mycobacteria for milk fat, which is more efficiently removed in the enzymatic method. About the same results were obtained with the DEFT and ChemScan methods, while the BactoScan showed a considerable variation because the intensity of the pulses approached the discrimination level of the apparatus. This is probably due to the specific cell wall composition of mycobacteria. In one experiment there was a 5 times underestimation, in a second experiment the underestimation increased with the concentration from 6.6 to 20. The PCR enzymatic based detection reached an average sensitivity of 30 cfu/ml. These results indicate that *M. paratuberculosis* cells could be efficiently detected in raw milk by the enzymatic based PCR method. Although no specific detection was achieved with the ChemScan using the above viability labelling, it could be promising for the automatic and accurate enumeration of *M. paratuberculosis* in milk.

Title Detection of *Mycobacterium paratuberculosis* in milk from infected cows by PCR and culture.

Author(s) Giese SB, Ahrens P.

Institution Danish Veterinary Laboratory, Copenhagen, Denmark

Abstract We started out spiking milk samples from cows tested negative for paratuberculosis. This was in order to establish the PCR and cultivation procedures and also to investigate in which milk fractions (cream, pellet or whey) *M. paratuberculosis* was found in largest numbers. Our results showed bacteria in pellet and cream but hardly any in whey. After this we sent out tubes for milk sampling to practitioners if faeces samples from their patients had shown the presence of acid fast bacilli. The practitioner was asked to take four milk samples (one from each quarter after a proper outer disinfection of the udder) from each cow. When we got the tubes back we spun down the milk and performed culture (directly on solid medium and in Dubos broth for 2 weeks followed by culture on solid medium) and PCR on both the pellet and the cream fractions. Of 34 sets of tubes sent out, we received 12 sets back at the laboratory. *M. paratuberculosis* was cultivated in innumerable numbers from 8 of these 12 animals faeces or intestinal mucosa. From 5 cows milk (all faeces culture positive) we cultivated *M. paratuberculosis* in few numbers (less than 10 colonies pr. tube) and milk from 2 cows were PCR positive (both animals were faeces culture positive and 1 cow was milk culture positive). One cow was culture negative on intestinal mucosa but culture positive in milk and 3 cows were negative in culture and PCR from both faeces and milk. *M. paratuberculosis* was equally found in pellet or cream. In conclusion: We can show the presence of *M. paratuberculosis* in milk by PCR but cultivation of milk is more sensitive.

Title The Development of a Laboratory Accreditation Program for Johnes Disease.

Author(s) Payeur JB.

Institution USDA, APHIS, NVSL, Ames, IA,USA.

Abstract As part of the voluntary National Paratuberculosis Certification Program developed by the United States Animal Health Association - Johnes Committee, the National Veterinary Services Laboratories (NVSL) were asked to develop a program to approve laboratories to perform tests for the diagnosis of Johnes Disease. Guidelines have been developed for antibody-based and organism-based tests. Laboratories are evaluated on their ability to perform the ELISA, although test performance can be provided for the complement fixation (CF) and AGID tests if requested. Faecal culture is the standard for the organism-based test although DNA probe tests are also evaluated if requested. The test panels consist of sera and faecal samples from known naturally infected culture positive cattle and known culture negative cattle herds. An approved laboratory must pass an annual check test to maintain its status. If a laboratory fails to meet the established criteria, this does not prevent them from performing the tests since Johnes Disease is not an official USDA disease control or eradication program at the present time. However, the national program guidelines recommend that each state only send samples to NVSL approved laboratories. Efforts are also being made to standardise the faecal culture methods and develop quality control guidelines for laboratory procedures and media.

Title Quality Assurance for Johnes Disease Testing.

Author(s) Condron RJ, Hope AF, Walker S.

Institution Victorian Institute of Animal Science, Attwood, Australia.

Abstract Laboratory testing is pivotal to national Johnes disease programs developed by the Australian livestock industries. The Sub Committee for Animal Health Laboratory Standards (SCAHLs) has established standard diagnostic techniques and implemented a proficiency testing program through the Australian National Quality Assurance Program (ANQAP) for veterinary laboratories in Australia and New Zealand for a range of diseases. More recently SCAHLs has developed a Quality Plan for Johnes disease testing to enable the application of a national operation standard for laboratories performing Johnes disease diagnostic tests and for manufacturers supplying diagnostic reagents. The Quality Plan is based on standard diagnostic techniques, proficiency testing of laboratories and guidelines for assessing the performance of serological tests. The plan identifies issues which impact on test quality and describes actions required to maintain and promote a quality operating system. Implementation of the Quality Plan was initiated at an ANQAP Quality Assurance Workshop held in April 1998. Groups with particular responsibilities in the Quality Plan include; testing laboratories, ANQAP and manufacturers of diagnostic reagents. ANQAP conducts proficiency testing for serological tests and culture of faeces by distributing reference and 'unknown' samples, analysing results and publishing an Endorsed List of Laboratories. National serum panels are being assembled to facilitate objective and comparative assessment of the reproducibility of serological assays. Criteria have been established to monitor the performance of assays by diagnostic laboratories, kit manufacturers and ANQAP. It is envisaged that the Quality Plan will be reviewed regularly to enable continuous improvement of this operating system.

Title Australian National Quality Assurance Program.

Author(s) Walker S, Rohde M, Condron RJ.

Institution Victorian Institute of Animal Science, Attwood, Australia.

Abstract The Australian National Quality Assurance Program (ANQAP) facilitates quality assurance in twenty government and private laboratories in Australia and New Zealand. ANQAP focuses on interlaboratory proficiency testing of assays used in quarantine, export certification and national health programs. Currently ANQAP evaluates forty serological assays and includes three

bacteriological quality assurance programs, the culture and identification of *Mycobacterium bovis* (WA) and *Mycobacterium paratuberculosis* (VIAS) and Footrot gelatin gel interlaboratory testing (WA). Participants of the serological testing program receive national reference antisera and a panel of "unknown" sera for use in the blind test appraisal. Results are supplied to ANQAP for a split level analysis based on a robust statistical procedures. Raw results and interpretation are compared to consensus mean values. Laboratories which report acceptable results are identified on the ANQAP Endorsed List of Laboratories. Laboratories which have reported unacceptable results due to incorrect interpretation or significant variation from the expected result are identified as unacceptable on the Endorsed List. These laboratories are required to undertake an internal investigation with the assistance of ANQAP to identify and rectify the test problem. Laboratories have the opportunity to undertake Re-endorsement Testing to be reinstated on the Endorsed List. As well as proficiency testing ANQAP assists in the training of laboratory staff, disseminates information and conducts workshops: assists in the development and revision of Australian Standard Diagnostic Techniques; provides positive and negative controls and panels of sera for in-house test validation; and provides advice on general laboratory quality assurance and external certification and accreditation.

Title	A Four-Laboratory Study to Assess Reproducibility in the IDEXX Johne's Antibody Test Kit.
Author(s)	Jacobson RH, Byrum B, Whitlock RH, Stabel JR.
Institution	Cornell University, Ithaca, New York; Ohio Department of Agriculture, Reynoldsburg, Ohio; University of Pennsylvania, Kennett Square, Pennsylvania; National Animal Disease Center, Ames, Iowa.
Abstract	<p>Each of four laboratories conducted three runs of the IDEXX Johne's Antibody assay. Each run consisted of two plates: one was from a kit lot used at all laboratories, and the other from a kit lot unique to each laboratory. A panel of 10 sera was tested in duplicate in all plates at each laboratory. Also, 108 sera were chosen and run by each lab with 1/3 of the sera tested in duplicate in each of the 2 plates per run. Thus, 432 sera were used to evaluate between-duplicate variability, and ten serum standards were used to assess variability between runs of the assay as well as between kit lots within the same run of the assay. For the kit lot used among all labs, the between-duplicate median coefficient of variation (CV) for the 432 samples was 3.4% (range between labs was 2.6% to 4.9%). The overall between-run CV, based on optical density (OD) readings of the 10 standards, averaged 17.3% (range between labs was 4.1% to 27.6%). Such variation in raw ODs between runs is not unusual in ELISA. Accordingly, ODs are usually adjusted (normalised or indexed) to a serum control(s), reducing the variation between runs. In the IDEXX assay, a ratio $[(\text{sample OD} - \text{neg control OD}) / (\text{pos control OD} - \text{neg control OD})]$, is calculated for this purpose. When this was done, the variability unexpectedly increased to a mean CV among laboratories of 22.0% (range = 11.1% to 31.7%; outliers not included to reduce bias) for the 10 serum standards. The negative control OD was eliminated from the calculation of ratios. The CVs for the resultant S/P ratios (18.6%) indicated that factors other than the negative control were responsible for the variation. Since the positive control had low activity (mean OD of 0.320), one of the 10 standard sera (mean OD of 1.288) was substituted for the positive control in the S/P calculation to reduce the impact of a potentially large systematic and/or random error factor. Unfortunately, this did not change the CVs significantly. The IDEXX Johne's antibody assay is variable, resulting in possible misclassification of animals having ratios near the assay's cutoff. The extent of this potential problem currently is being determined by evaluating over 10,000 results from routine runs of the assay. These data will be discussed.</p>

Title Immodulation of early events during experimental Paratuberculosis.

Author(s) Begara-McGorum I, Wildblood LA, Stevenson K, Sharp JM, Jones DG.

Institution Moredun Research Institute, International Research Centre, Pentland Science Park, Bush Loan, Penicuik EH26 0PZ Scotland, UK.

Abstract Recently, we described changes in lymphocyte distribution and function, total and specific mRNA expression in gut-associated lymphoid tissue (GALT) of neonatal lambs experimentally infected with a cervine strain of *Mycobacterium avium* subspecies paratuberculosis (Map). In this study, a similar protocol was used to assess immune events in vaccinated and non-vaccinated lambs, and to compare responses between animals infected with cervine or ovine isolates of Map. Five groups of five lambs were treated as follows: A) Non-infected/non-vaccinated (NINV); B) Non-infected/vaccinated (CVL, Weybridge Johne's disease vaccine) (NIV); C) Infected (cervine isolate)/non-vaccinated (CINV); D) Infected (cervine isolate)/vaccinated (CIV); E) Infected (ovine isolate)/non-vaccinated (OINV). Findings in A and B were comparable to those from the original experiment: circulating IgG and GALT B cell numbers were significantly ($p < 0.05$) decreased and GALT T/B cell ratios increased. Vaccination and/or infection with the ovine strain had a significant ($p < 0.05$ by ANOVA) impact on both lymphocyte and Ig distribution. The relevance of these early immune changes and their regulation in terms of disease development is under further investigation.

Title Local and systemic immune response after vaccination against *Mycobacterium avium* subsp. paratuberculosis in goats.

Author(s) Valheim M, Press C McL, Hasvold H, Larsen HJ.

Institution Norwegian College of Veterinary Medicine, Oslo, Norway.

Abstract Vaccination of goat kids against paratuberculosis provides protection against clinical disease, but the nature of the cellular reaction in lymphoid tissue has not been described. Fourteen of 28 male kids were vaccinated subcutaneously cranial to the scapula with a commercial paratuberculosis vaccine (Paratuberkulosevaksine, Central Veterinary laboratory, Oslo, Norway). After three weeks, seven of the vaccinated and seven of the control goat kids were euthanased. Tissues from the vaccination site and the draining prescapular lymph node were fixed in formalin or frozen. The remaining seven vaccinated goat kids received a subcutaneous injection of a pathogenic strain of *M. avium* subsp. paratuberculosis close to the vaccination site. Three weeks after the challenge, these seven goat kids and the remaining seven control animals were euthanased and the tissues were collected as for the previous groups. Blood samples were collected at various times throughout the experiment and humoral and cellular immune responses were detected in the blood 12 weeks after vaccination. Prescapular lymph nodes from all the goat kids were subjected to bacteriological examination. Histological examination showed that there was a granulomatous reaction at both the vaccination site and in the draining lymph node. ZN staining for acid fast bacilli and immunohistochemical examination for *M. avium* subsp. paratuberculosis showed reactivity at the vaccination site three weeks after vaccination but not at 12 weeks and bacteria were not detected in the draining lymph node at either time of examination. There was a significant follicular hypertrophy in the lymph node but significant changes in the proportion of area occupied by T-cell subpopulations in the diffuse lymphatic tissue of the lymph node cortex were not detected. The results of the present study suggest that large changes in T-cell subpopulations in the lymph node cortex does not characterise the successful immune response of goats vaccinated against *M. avium* subsp. paratuberculosis.

Title Immune Response of Goats Experimentally Infected with *Mycobacterium avium* ss paratuberculosis.

Author(s) Larsen HJS, Storset AK, Hasvold H, Knagenhjelm SKH, Berntsen G, Brun-Hansen H, Holstad G.

Institution Norwegian College of Veterinary Medicine, Oslo, Norway.

Abstract An experimental oral infection of goats with a caprine isolate of *Mycobacterium avium* ss paratuberculosis was used to investigate immunological and bacteriological changes during the first year post-infection. Seven 2-3 months old goats were given a bacteria-suspension in milk three times weekly for nine weeks. Six animals were kept as controls. The lymphocyte response following in vitro stimulation with specific antigens was characterised using the lymphocyte proliferation test, the gamma-IFN immunoassay and the IL-2 receptor test (flow cytometry). The antibody response was assayed using an ELISA technique. Lymphocytes from infected animals responded significantly within nine weeks in all three tests, and still do one year after infection. Four infected animals showed a weak serological response as early as 15 weeks post infection and all were serological positive after one year. The bacterial examination of the faeces was negative during the first year post infection.

Title Characterisation of in vitro stimulated cells from *Mycobacterium avium* ss.paratuberculosis infected goats

Author(s) Storset AK, Berntsen G, Larsen HJS.

Institution Norwegian College of Veterinary Medicine, Oslo, Norway.

Abstract To quantify cellular immunity in ruminant paratuberculosis, parameters like gamma-IFN release and lymphocyte proliferation have been measured after in vitro stimulation with specific antigen. In this study we describe the quantification of surface interleukin 2 receptor (IL-2R), an early marker of lymphocyte activation and a prerequisite for lymphocyte proliferation, after in vitro stimulation with PPD Johnin. In order to characterise the phenotype of the lymphocytes expressing IL-2R, cells were processed for dual- or three colour analysis by flow cytometry. Blood collected from *Mycobacterium avium* ss.paratuberculosis inoculated and healthy goats were diluted 1:10 in RPMI and stimulated with 10 mg/ml PPD Johnin for 72 hours. Non-stimulated control cultures were included. Erythrocytes were hypotonically lysed and leukocytes stained with monoclonal antibodies against IL-2R and CD4 or IL-2R, CD8 and gamma-delta-T cells using FITC, PE and Chycrome conjugated secondary antibodies followed by flowcytometric analysis. Small lymphocytes and lymphoblasts were distinguished by their forward/side scatter characteristics. In both regions the population of IL-2R expressing cells were determined as CD4+, CD8+, gd+ or CD8+/gd+ . At ten months post oral infection, the percentage of IL-2R expression of small lymphocytes and lymphoblasts were significantly higher in the infected group of animals than in the control group. In the lymphoblast region, there were significantly more IL-2R positive cells expressing CD4+ and CD8+ among the infected animals, while the main population of IL-2R expressing cells in the control group were of the gamma-delta-T cell subset. In the region of small lymphocytes there were significantly more IL-2R cells expressing CD8+ among the infected animals.

Title Cytokine mRNA expression and uptake of *Mycobacterium avium* subspecies paratuberculosis by sheep alveolar macrophages.

Author(s) Begara-McGorum I, Connor KM, Jones DG.

Institution Moredun Research Institute, International Research Centre, Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ

Abstract To investigate the influence of *Mycobacterium avium* subspecies paratuberculosis (M.a.paratuberculosis) on cell-mediated immunity through the production of cytokines, ovine alveolar macrophages (AM) were infected in vitro with M.a.paratuberculosis and production of cytokine mRNA by infected AM was assessed at different time points. mRNAs encoding IFN-gamma, IL-1 β , IL-4, IL-6 and TNF-alpha were expressed, although the kinetics of expression varied. Transcription of GM-CSF, IL-2 and IL-12 was not detected. Studies on the uptake of M.a.paratuberculosis by AM, and a comparison with ingestion of a non-virulent mycobacterial strain *M.phlei* were also performed. While uptake of M.a.paratuberculosis by AM increased significantly with incubation time, uptake of *M.phlei* increased at a much slower

rate, resulting in a significantly increased ($p < 0.05$) infection index for *M.a.paratuberculosis* at 24h and 48h compared with *M.phlei*. Electron microscopic studies of AM confirmed differences in the pattern of ingestion of the two mycobacteria species. This study adds to our understanding of the complex interaction between *M.a.paratuberculosis* and their host cells and could help to clarify the cytokine network during paratuberculosis. The predominance of induction of proinflammatory cytokines by *M.a.paratuberculosis* and the failure to induce GM-CSF and other Th1 cytokines could be of relevance for the pathogenesis of the disease.

- Title** Comparative cytokine profiles in ruminant paratuberculosis (Johne's disease).
- Author(s)** Uilenreef JJ¹, Stewart DJ², Rutten VPMG³, Koets AP³, Scheerlinck JP¹, Prowse SJ².
- Institution** ¹ CRC for Vaccine Technology Unit, CSIRO Division of Animal Health, Private Bag No 1, Parkville, Victoria 3052, Australia. ² CSIRO Animal Health, Australian Animal Health Laboratory, Private Bag No 24, Geelong, Victoria 3220, Australia. ³ Dept. of Immunology, Inst. of Inf. Diseases and Immunology, Faculty of Veterinary Medicine, Yalelaan 1, Postbus 80163,3508 TD Utrecht, The Netherlands.
- Abstract** In any control program aimed at eradicating *M. avium* subsp. paratuberculosis, the detection of infected animals in the preclinical stage is vital, since shedding and therefore transmission of the bacteria occurs before the onset of clinical symptoms. The measurement of the Cell Mediated Immune response by detection of IFN-g following antigen specific stimulation of lymphocytes is one of the most promising approaches to detect subclinical infected animals. Using Enzyme Immune-Assays, 7 cytokines (IL-1 β , IL-2, IL-6, IL-8, TNF-alpha, GM-CSF and IFN-gamma) were measured in whole blood culture supernatants derived from control and infected animals¹. The cultures were stimulated with either *M. avium* PPD, Johnin PPD or were left unstimulated. Of the cytokines investigated to date in the lambs and kids, IFN-gamma was found to correlate closest to infection status, especially when presented as a 'classical' stimulation index (ratio between cytokine responses of stimulated versus non-stimulated cells). In the calves however, the IFN-gamma responses of the control group were similar to the infected groups¹. The IFN-gamma responses in the controls may be due to the calves being sourced from an unknowingly infected farm. In all three species IL-1 β responses of the infected groups were following the same trend as those of IFN-g, albeit hardly distinguishable from the controls. For the TNF-alpha, GM-CSF and IL-8 responses, no difference between the control and infected groups in either species could be found. IL-2 and IL-6 could not be detected, which could be due to having a much higher detection limit than the IFN-gamma assay and using a suboptimal incubation period for cytokine expression respectively. This data suggests that IFN-gamma is to be preferred over the other cytokines as a marker for infection with *M. paratuberculosis*, but further optimisation of some of the assays may reveal other suitable markers for infection. This work is in progress and continuing.

- Title** Lewis rats are not susceptible to oral challenge with *Mycobacterium paratuberculosis*.
- Author(s)** Koets AP¹, Rutten VPMG¹, Bakker D³, van der Hage MH², van Eden W¹.
- Institution** ¹ Institute of Infectious Diseases and Immunology and ² Department of Pathology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands, and ³ Institute for Animal Science and Nutrition, Lelystad, The Netherlands.
- Abstract** Pathogenesis studies of *M. paratuberculosis* infection in ruminants are hampered by the long incubation time of the disease. Although small rodents are usually considered to be resistant to *M. paratuberculosis* infection, several murine strains with higher susceptibility have been found. There are no detailed reports with regard to susceptibility in rats. The Lewis rat is a valuable model for inflammatory bowel disease studies as well as immune mediated disorders involving mycobacteria as inducing agents. Thus we decided to use the Lewis rat to investigate its potential as a small laboratory animal model for paratuberculosis. In total 24 female Lewis rats were orally inoculated with *M. paratuberculosis*. The rats were first inoculated at 3 weeks of age, and 12 more inoculations followed in a decreasing frequency during the 3 months to follow. Twelve control rats received a sham inoculation. At regular intervals rats from each

group were sacrificed and immunological and histopathological examinations were performed on the gastrointestinal tract, the liver and the spleen over a nine month period. None of the rats developed lesions which were indicative of mycobacterial infection as determined by histology with HE and Ziehl-Neelsen staining. The bacteria could not be recultured from samples taken from the gut, the liver or the spleen. Some of the immunological tests however, showed evidence that bacteria had entered via the intestinal tract. We conclude that Lewis rats are resistant to oral challenge with *M. paratuberculosis*, and can not serve as a model in studying the immunopathogenesis of paratuberculosis.

Title Shedding mechanism of *M. avium* subspecies paratuberculosis from intestinal mucosa.

Author(s) Momotani E¹, Yamaguchi M², Tanaka K¹, Arita Y¹, Iga M¹, Yoshihara K¹.

Institution ¹ Laboratory of Molecular Pathology, National Institute of Animal Health 3-1-1, Kan-nondai, Tsukuba 305-0856 and, ² Tokachi Livestock Hygiene Service Center, Hokkaido, Japan

Abstract *M. avium* subspecies paratuberculosis causes bovine paratuberculosis and suspected to be possible participation to human Crohn's disease. Immunological diagnosis is ineffective in anergic state in the long incubation period (IP), for 3 to over 6 years, however, bacterial isolation from faecal sample is a reliable diagnostic method in the anergic state. So many bacteria/day are shed into faeces in the clinical and subclinical stages. However, no evidence of the shedding mechanism of the bacteria in the granulomas to intestinal lumen, through the mucosal epithelial barrier. We report here that novel passive shedding mechanism by using host macrophages that treat apoptotic epithelial cells in constant epithelial renewal. These results and fact that they enter through M-cells, strongly emphasise the successful nature of the bacterial that invade and escape passively by using host physiological function without any damage.

Title Role of Nramp 1 in Johne's Disease.

Author(s) Beard P¹, Hopkins J², Rhind S², Stevenson K¹, Sharp JM¹.

Institution ¹ Moredun Research Institute, International Research Centre, Pentland Science Park, Bush Loan, Penicuik EH26 0PZ. Scotland, UK. ² Department of Veterinary Pathology, University of Edinburgh, Summerhall, Edinburgh EH9 1QH Scotland, UK.

Abstract Genetic factors are hypothesised to influence susceptibility to paratuberculosis, but as yet, no specific genes have been identified that correlate with either disease incidence or pathological changes. Nramp 1 is a highly conserved gene thought to encode a protein which performs an as yet unknown function in the membrane of phagosomes in macrophages. A single base mutation in Nramp 1 has been found in certain strains of mice. Animals with the mutated allele show increased susceptibility to intracellular pathogens such as *Salmonella typhimurium*, *Leishmania donovani* and BCG. Our work focuses on the effect of Nramp 1 on paratuberculosis, as we screen Johne's affected and non affected animals for the presence of this mutation, and determine its effect on the incidence and progression of paratuberculosis.

Title Immunological and bacteriological time course studies on experimental *Mycobacterium paratuberculosis* infections in sheep, goats and calves.

Author(s) Stewart D, Vaughan J, Noske P, Jones S¹, Tizard M, Prowse S.

Institution CSIRO Animal Health, Australian Animal Health Laboratory, Private Bag 24, Geelong, 3220 Australia and ¹ CSL Limited, 45 Poplar Road, Parkville, 3052 Australia.

Abstract Weaned lambs, kids and calves (5 animals per group) were experimentally infected with either cultured bovine *Mycobacterium paratuberculosis* or organisms in intestinal mucosal scrapings from naturally infected cows at 6 months, 5 months and 6 weeks of age, respectively. Four doses of cultured cells (1×10^{10} or 2×10^{10} per ml) or mucosa (15 to 20 g) were given orally at

weekly intervals for 4 weeks. Control lambs, kids and calves (5 animals per group) were dosed with broth diluent. Blood samples for the bovine gamma interferon (IFN-gamma) test (Bovigam®, CSL Limited) and the Johne's absorbed EIA (Parachek®, CSL Limited) and faecal samples for conventional culture and radiometric culture (BACTEC®, Becton Dickinson) were taken pre-challenge and monthly post-challenge. Whilst the experiment is still in progress, these are the results up to five months post-challenge. All lambs in the mucosa dosed group and 2/5 of the organism dosed group became culture positive at 2 mo. Only 1 lamb in the mucosa dosed group remained culture positive at the 3 and 4 months sampling. At 2 mo, 3 of the lambs in the mucosa group had elevated avian PPD IFN-gamma responses. At 3 to 5 mo all of the challenged lambs had positive avian PPD IFN-gamma responses. There were 3, 4 and 2 culture positive kids at 2, 3 and 4 mo, respectively with 2 kids shedding for at least 2 sampling periods. Avian PPD IFN-gamma responses became evident at 3 mo. Both lambs and kids challenged with mucosa generated higher IFN-gamma responses. Johnin PPD generally gave higher IFN-gamma responses than avian PPD. No IFN-gamma responses were detected in the lamb and kid controls. Three calves in the mucosa group at 3 and 4 mo were culture positive. Interestingly, challenged and control groups of calves had similarly high avian PPD IFN-gamma responses. The IFN-gamma responses in the controls may be due to the calves being sourced from an unknowingly infected farm. Of all the animals tested, only 2 kids were antibody positive in the absorbed EIA at 4 and 5 mo. The data suggests that the IFN-gamma response may be used for the early detection of Johne's disease in sheep.

Title	Relation between ovine paratuberculosis lesions and cellular and humoral immune responses in diagnostic tests.
Author(s)	García Marín JF, Tellechea J, Corpa JM, Gutiérrez M, Pérez V.
Institution	Departamento de Patología Animal: Medicina Animal (Anatomía Patológica). Facultad de Veterinaria. Universidad de León. Campus de Vegazana, s/n. 24071 León (Spain).
Abstract	An immunopathological spectrum which correlates pathological forms with immune responses has been described in mycobacteriosis such as leprosy or tuberculosis. In paratuberculosis, a relationship between the response to immune based diagnostic tests and different types of lesions have been also previously reported. In this paper, the possible association between diagnostic tests and the histological lesions observed in sheep (focal or subclinical and diffuse or clinical) is studied. Cellular immunity was assessed by means of gamma-IFN and intradermal skin test and AGID and ELISA were used as serologic tests. A total of 134 sheep were evaluated by pathological methods and lesions classified as focal (located only in the lymphoid tissue), multifocal or diffuse. The latter were divided in two types according to the cellular types and the number of bacilli (borderline tuberculoid-borderline lepromatous). Focal lesions were usually negative to serology and positive to cellular tests. Among diffuse lesions, borderline-lepromatous type (with high amounts of bacilli) were positive to serology and negative to cellular tests whereas borderline-tuberculoid lesions gave the opposite reactions. Multifocal forms gave variable responses. The simultaneous use of cellular and humoral immunity based tests can detect the majority of infected animals. So, both groups of tests have to be regarded as complementary and their use should be considered in control eradication programs based on immunological diagnostic tests.

Title	Sequential antibody response of calves to Mycobacterium paratuberculosis infection using Western blotting.
Author(s)	Ganci DA, McDonald WL, Hope A.
Institution	Victorian Institute of animal Science, Attwood, Australia.
Abstract	Western blots of sera from calves infected with Mycobacterium paratuberculosis demonstrated an early antibody response that has potential diagnostic applications. Antibody responses in 13 calves at 6, 12, 18 and 24 months of age and of known M. paratuberculosis infection status were investigated. These included 4 uninfected calves born to test negative dams obtained from farms with a low prevalence of Johne's disease, 3 naturally infected calves born and raised by cows

shedding *M. paratuberculosis* in their faeces and 6 artificially infected calves which were either orally dosed with a field isolate of *M. paratuberculosis* or dosed with intestinal mucosa from a clinically infected cow. A sonicated cell lysate of *M. paratuberculosis* was separated by 10% SDS-PAGE and transferred to nitrocellulose membrane for Western blotting. Antibody reactions to the separated antigens were detected using anti-bovine IgG (whole molecule) alkaline phosphatase conjugate. Multiple reactions to antigen bands within a molecular weight range of 12 kDa to 162 kDa were observed in the sera of infected and uninfected calves. Each calf reacted to between 4 and 13 antigen bands. Bands which were specific and only recognised by serum antibodies of infected calves increased in number over time from 7 bands at 6 months, to 16 bands at 12 months, 35 bands at 18 months and 25 bands at 24 months. The number of non-specific antigen bands (common to sera of both test groups as well as unique to sera of uninfected calves) totalled 57% of all bands observed on the blots and did not increase over time.

- Title** Analysis of the antibody response by immunoblot and ELISA in sheep infected with *Mycobacterium paratuberculosis*.
- Author(s)** Kittelberger R¹, Meynell RM¹, Gwózdź JM², Reichel MP¹.
- Institution** ¹ Central Animal Health Laboratory, MAF Quality Management, Wallaceville, P.O. Box 40063, Upper Hutt, New Zealand. ² Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand.
- Abstract** The aim of this study was to identify possible immuno-dominant and *M. avium* spp. paratuberculosis-specific antigenic components in ovine paratuberculosis by analysing large numbers of sheep sera by immunoblot. Sera were used with and without pre-absorption with a *M. phlei* sonicate. The sera comprised serial bleeds from 44 sheep, experimentally infected with *M. paratuberculosis*, sera from lesion-confirmed, naturally *M. paratuberculosis*-infected sheep, and three sera groups from sheep free from paratuberculosis, namely 355 sera from Australia and 156 sera from the Falkland Islands. All sera were also tested in an absorbed ELISA. With sera from experimentally infected sheep protein bands of 47, 37, 30, 24 and 21 kDa and a broad band of a polysaccharide of 32 to 42 kDa were detected. A few other bands appeared infrequently, either of low molecular weight below 21 kDa or of high molecular weight around 100 kDa. Altogether, sera from 37 sheep stained bands in blots during the course of infection with variable frequencies. Sera from naturally infected, lesion-confirmed sheep stained the same bands as observed with the experimental sera. Some staining of bands was also seen with negative sera but most of these bands could be absorbed out. The results of this study indicate that the 32-42 kDa polysaccharide is immunodominant and shows a certain degree of specificity, while protein antigens appear to be less specific.

- Title** Microbiological and immunological characteristics of a cattle herd affected by paratuberculosis.
- Author(s)** Toman M, Pavlík I, Faldyna M, Matlova L, Bartl L, Svastova P, Horin P¹.
- Institution** Veterinary Research Institute, Brno; ¹ University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.

Abstract Prevalence of paratuberculosis has been monitored in a herd with 1700 cows for a long period. All animals have been examined serologically by AGID and CFT since 1990 and also by culture of faecal samples twice a year since 1993. *M. avium* subs. paratuberculosis isolates were identified by subculture and PCR (probe IS 900). Positive by faecal culture at the beginning of regular examination was 5.6% animals. There were relatively a lot of animals (23.1% from slaughtered cows) with only one positive finding in a faeces during the period of investigation and with negative results of pathological and microbiological examinations of intestines and mesenteric lymph nodes after slaughter. This animals, although infected, was considered resistant. Animals shedding repeatedly *M.a.* paratuberculosis in faeces (22.2% were low shedders, 28.2% were high shedders) and with findings after slaughter, were considered as susceptible. They were divided into the subgroups according their clinical status. In 1997 - 1998, the selected animals were tested immunologically. The set of techniques included flow cytometry, phagocytosis and chemiluminescence tests, lymphoblast-transformation test after stimulation with non-specific mitogens and PPD antigen of *M. avium* and determination of IFN-gamma after the stimulation of cells with specific antigen. The number of serologically positive animals increased with growing intensity of shedding of *M. a.* paratuberculosis, but animals positive by serological tests and negative by culture were also found. Positive results of cell-mediated immunity test, in particular the production of IFN-gamma, were obtained mostly in the animals classified as resistant. As to the parameters of non-specific activity of the immune system, larger differences were found between resistant and susceptible animals, than among subgroups of susceptible infected animals at various stages of the disease. Increased phagocytic activity was found in most animals affected by diarrhoea, however. The counts of lymphocytes and counts of the CD8+ cells were significantly lower in the subgroup of susceptible animals at an early clinical stage than in the subgroup of resistant animals with a minimal shedding of mycobacteria. (Supported by GACR 524/97/O948).

Title Effect of recombinant IL-12 administration on early immune response to vaccination for Johne's disease in calves.

Author(s) Chilton PM, Whitlock RH, Habecker P, Scott P, Sweeney RW.

Institution University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA USA.

Abstract Holstein calves were divided into 4 experimental groups: uninfected-unvaccinated controls, unvaccinated-infected, vaccinated-infected, and vaccinated+IL-12 -infected. Vaccinated calves were given commercially available killed *M. avium* Strain 18 (Mycopar®, Solvay) at 1 week of age by subcutaneous injection. Calves in the vaccine + IL-12 group were given 10 µg human recombinant IL-12 subcutaneously as a separate injection adjacent to the vaccination site. Three weeks after vaccination, calves in infected groups were given an oral challenge of 8×10^9 CFU of field isolate *M. paratuberculosis*, suspended in milk, once daily for 2 days. Calves were euthanased 3 weeks after oral challenge. Peripheral blood lymphocytes (PBL), prescapular lymph node cells (draining vaccination site), caecal lymph node cells, and spleen cells were cultured in-vitro with *M. paratuberculosis* antigen. Lymphoproliferation was measured by 3H-thymidine incorporation, and IFN-gamma concentration in culture supernatants was measured by ELISA. Within 3 weeks after vaccination, peripheral blood lymphocytes from vaccinated calves had 3- to 10- fold higher IFN-gamma production compared with unvaccinated calves. Oral challenge without vaccination resulted in no detectable increase in IFN-gamma production by PBLs. Caecal lymph node cells from calves vaccinated+IL-12 produced 4-fold greater concentrations of IFN-gamma than those calves vaccinated without IL-12, 15-fold greater than unvaccinated-infected animals, and 60-fold greater than uninfected-unvaccinated controls. Recombinant IL-12 given with Johne's disease vaccine may enhance the gut associated lymphoid tissue response to *M. paratuberculosis*.

Title Vaccination against paratuberculosis of lambs already infected experimentally with *Mycobacterium paratuberculosis*.

Author(s) Gwóźdz JM, Manktelow BW, Murray A, West DM, Thompson KG.

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Abstract Twenty eight lambs, aged 1.0-1.5 months, were inoculated orally with approximately 4.4×10^8 *Mycobacterium paratuberculosis* (*M. paratuberculosis*) organisms. Two weeks later 14 of these 28 animals were injected subcutaneously with 1.0 ml of a live-attenuated vaccine. Thirteen lambs were used as negative controls. Production of gamma-interferon (IFN-gamma) in blood, and antibody levels in serum were sequentially monitored in each sheep. The infection status of each sheep was determined by histology and an IS900-based PCR test on samples of ileum and ileocaecal lymph node at the time of necropsy. Between the 27th and 53rd week postinfection, 7 infected/unvaccinated (unvaccinated) and 2 infected/vaccinated (vaccinated) animals developed clinical signs consistent with paratuberculosis. In vaccinated sheep, an initial rise in both the IFN-gamma response and antibody titre was observed in the 5th week postinfection, while in unvaccinated sheep, an increase in IFN-gamma response was not detected until 18 weeks postinfection, and no increase in antibody level was detected until 27 weeks. Between the 5th and 53rd week postinfection, both the IFN-gamma and antibody concentrations were greater in the vaccinated group than in the unvaccinated group. The granulomatous inflammation in the jejunal and ileal mucosa was less severe in the group of vaccinated sheep than in the group of unvaccinated sheep. Acid fast organisms were detected only in the latter group. The PCR assay on ileal samples gave positive reactions in 2 vaccinated and 8 unvaccinated sheep. These data indicate that vaccination of lambs with live-attenuated vaccine 2 weeks after oral inoculation with *M. paratuberculosis* stimulated the host response against the organism and led to a reduced mycobacterial burden. Furthermore, the mean IFN-gamma production was higher in 7 clinically normal unvaccinated sheep than in 7 unvaccinated sheep with clinical paratuberculosis. This suggests a positive relationship between the magnitude of peripheral cell-mediated response and ability to control the infection.

Title The T1 to T2 shift in bovine paratuberculosis and the role of activation induced cell death.

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Abstract Evidence is accumulating that the progressive aspects of bovine paratuberculosis are related to a shift from a T1, cell mediated, to a T2, humoral, type of immune response. We have studied the cellular and humoral responses in paratuberculous cattle to a number of mycobacterial antigens regarding the T1 to T2 shift during the progression of the disease, and the role of activation induced cell death (AICD) in this switch. In 150 animals in different stages of paratuberculosis lymphocyte proliferation assays and ELISA were used to evaluate responses to different PPD's, the 65 and 70 kD recombinant mycobacterial heat shock proteins, and lipoarabinomannan (LAM) antigens. In an additional 20 animals local gastrointestinal responses to the same antigens using the same techniques, in addition cytokine mRNA levels were evaluated and compared to what was found in peripheral blood. In vitro cultures of (un)infected bovine monocyte derived macrophages and T cells, and the TUNEL assay were used to evaluate AICD. The results indicate that a T1 to T2 shift appears to take place during the disease but that, especially with regard to the humoral responses, the type of antigen and its putative predominant localisation during the infection play a major role in both the direction and magnitude of the switch. Furthermore we have found indications that AICD of T cells caused by chronically infected macrophages in situ may be a possible mechanism for this T1 to T2 switch during bovine paratuberculosis.

Title T-cell response to *Mycobacterium paratuberculosis* proteins in Black Bengal Indian goats.

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Abstract Protective immunity in mycobacterial infection is predominantly mediated by antibody independent cellular response involving recognition of mycobacterial antigens by T lymphocytes. In the present study secretory proteins of *M. paratuberculosis* (strain TEPS) was subjected to fractionation through DEAE Sephacel column chromatography and the fractionated protein evaluated for their T-cell reactivity. Crude culture filtrate protein was also resolved by SDS-PAGE followed by transfer to NCP for T-cell blotting. Fraction eluted through the column was subjected to SDS PAGE and the fractions having similar banding pattern were grouped together. According to such protein profile five groups were obtained out of them only one group was homogeneous having a 40 KD. MW peptide. All these five fractions were subjected to in-vitro lymphoproliferation test and cytotoxicity test on Black Bengal Indian goats. Young goats from Johne's disease free herd were housed in two groups, group-1 was sensitised with crude culture filtrate protein and group-11 was inoculated with live bacteria. In-vitro lymphocyte transformation test was done on day 80 and day 120 of post sensitisation. Highest thymidine uptake was recorded with 40 KD. MW protein fraction whereas other fractions did not show such a high response. A slight upward trend in Lymphoproliferation was observed on day 120 of post sensitisation as compared to day 80 in both the groups of animals. Animals sensitised with live bacteria have shown much higher stimulation index (SI-68.7) with same 40 KD. polypeptide, the proliferative response was considerably higher in comparison to animal sensitised with protein antigen. Cell mediated immune response was found to be persisting up to a period of 310 days as observed by lymphoproliferation. On T-cell blotting experiment using peripheral blood mononuclear cells from live bacteria sensitised goats, the crude antigen has shown major lymphoproliferative response around 30-50 KD MW region. T-cell response below and above this region was relatively poor. However on in-vitro cytotoxicity assay, autologous monocycle targets pulsed with live bacteria have been found to be effectively lysed than that of target cells pulsed with fractionated protein antigens. Further study is in progress for in-vitro and in-vivo detection of CMI response in pre-clinical cases of paratuberculosis using the 40 KD MW protein antigen.

Title Interactions of *Mycobacterium avium* subsp. *paratuberculosis* with murine macrophages: Intracellular survival and modulation of macrophage functions.

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Abstract Murine macrophages were infected in vitro with *M. avium* subsp. *M. paratuberculosis*, strain 6783. Samples were taken at different time points up to 10 days to study intra-cellular fate of the bacteria by electron and confocal laser scanning microscopy. Results revealed that mycobacteria were taken up efficiently within 30 min. p.i. and persisted intracellularly in phagosomes for the whole infection period. Bacterial uptake and persistence was associated with a time dependent modulation of the expression of several cytokine and transcriptional control genes as determined by RT-PCR. Expression of the genes for IL-1 β , IL-6, GM-CSF and IL-12 was significantly enhanced, starting at 2 h p.i. and remaining up to 72 h p.i.. Interestingly, expression of the genes for TNFI and c-fos was not or only slightly modified. Mycobacterial persistence also resulted in a suppressed presentation of exogenously added antigen haemagglutinin (HA) as analyzed by measuring IL-2 production of HA specific T-cells. Reduced antigen presentation seemed to be caused by reduced processing of HA and was not accompanied by any alterations of the expression of MHCII and co-stimulatory molecules B7.1 and B7.2.

Title Parallel faecal and organ *Mycobacterium avium* subsp. *paratuberculosis* culture showing distribution in the body of an individual and DNA fingerprinting of isolated strains.

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Abstract Faecal and organ examinations were carried out in 611 animals originating from eight paratuberculosis infected cattle herds. The diagnosis in the 479 cattle was set according to faecal (at least 3 months before slaughtering) and routine intestinal culture (ileum and the adjacent lymph nodes) after slaughtering. In the remaining 132 animals, post-mortem detailed culture was performed and samples were collected from the GIT (duodenum, jejunum, ileum, ileocaecal valve, caecum, rectum and the corresponding lymphnodes), lymphnodes (around the head, udder, lungs and liver), liver and spleen. In 251 (41.1%) of the animals, M.a.paratuberculosis could be isolated from the faeces; in 164 (65.7%) out of 251 shedding animals the infection was detected in the ileum and adjacent lymphnodes. The number of M.a.paratuberculosis present in organs of infected animals varied from 46.0% shedding 1 CFU, to 94.7% with massive shedding (correlation coefficient $\chi=0.79$ and $\alpha=0.01$). In 92 (25.5%) of the 360 non-shedding animals, the infection was culturally detected in the ileal mucosa and corresponding lymphnodes. Shedding animals had significantly higher ($p=0.01$) number of organisms in their organs than the non-shedding. During the detailed cultivation of other organs from 132 infected animals, 72 (54.5%) of them were positive. In 16.7%, the infection was spread in the intestine, parenchymatous organs, lymphnodes in lungs and head region and in 9.7% the infection was detected around the lymphnodes of the head region or the lungs. Randomly isolated strains were determined by DNA fingerprinting using IS900 as probe with restriction endonucleases PstI and BstEII. Strains isolated from the faeces and organs of 150 infected animals were examined by DNA fingerprinting. Only six animals showed mixed infection caused by strains with different DNA types. Our research was partially supported by the Ministry of Agriculture of the Czech Republic (grant no. EP0960006087) and Czech Grant Agency (grants No. 514/95/1594 and 524/97/0948). Permanent address of Robin du Maine - Hogeschool van Utrecht, Netherlands.

Title Caprine paratuberculosis: gross and histological changes in the intestine and other tissues.

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Abstract Anatomopathological study was developed in 28 goats from 3 flocks naturally infected with Map. The goats that were positive to AGID, ELISA or both tests such as the ones that showed progressive weight loss were considered paratuberculosis infected and were necropsied. Jejunum, ileum, ileocaecal valve, liver and mesenteric lymph nodes fragments were collected and stained with H-E and Z-N stains. Discrete to severe folding of the mucosa was observed, the subserosal lymphatic vessels were prominent. The mesenteric lymph nodes were enlarged, oedematous and the corticomedullar distinction was poor in most goats. Microscopically, the lesions consisted in focal granulomatous lesions on Peyer's patches and associated mucosa with scarce AFB, zonal granulomatous enteritis with moderate to abundant AFB, and diffuse severe granulomatous enteritis with moderate and abundant AFB. In the mesenteric lymph nodes the lymphoid cells within the paracortical zone were discrete to largely replaced by macrophages and giant cells, scarce to abundant afb were observed; eventually, discrete foci of necrosis and mineralisation were present such as foreign body giant cells. Liver lesions consisted in periportal mononuclear infiltrate and one goat showed multifocal granulomatous hepatitis with foreign body giant cells and abundant AFB. The lesions above described agree with the ones reported in goats naturally infected with Map. Necrosis, caseation and ulceration as they occur in tuberculosis were always absent.