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Third International Colloquium on Paratuberculosis

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Title Serological and cellular assays for the diagnosis of mycobacterial infections in cattle.

Author(s) Wood PR, Billman-Jacobe H, Carrigan M.

Institution CSIRO Division of Animal Health, Parkville, Victoria 3052, Australia. NSW Department of Agriculture, Orange 2800, Australia.

Abstract A common feature of mycobacteria is they induce a wide spectrum of immunological responses in infected animals. We have therefore examined both the antibody response by ELISA and the T cell response using a gamma interferon assay in cattle infected with *M. bovis* or *M. paratuberculosis*. The predominant immune response in cattle is a cellular response and the interferon assay had a sensitivity of 93% for bovine tuberculosis and 73% for Johne's disease. In contrast, less than 50% of TB infected cattle had a detectable antibody response to an *M. bovis* culture filtrate antigen preparation. With Johne's disease infected cattle the level of humoral response as detected by the absorbed ELISA increased as the disease progressed. In subclinical animals not shedding bacteria in their feces, 16% were antibody positive, compared to 40% of subclinical fecal shedders, and 77% of animals with clinical disease. Because of this broad spectrum of immune responses, particularly with Johne's disease, we recommend that assays for both antibody and T cell reactivity be used in the control of Johne's disease in infected cattle herd.

Title Comparison of the Johne's absorbed EIA and the complement-fixation test for the diagnosis of Johne's disease in cattle

Author(s) Ridge SE, Morgan IR, Condron RJ.

Institution Victorian Institute of Animal Science, Attwood, Victoria, 3049, Australia. Sockett DC, Collins MT. University of Wisconsin, School of Veterinary Medicine, Madison, Wisconsin, USA

Abstract A commercially available absorbed ELISA for the diagnosis of Johne's disease in cattle, the Johne's Absorbed EIA, was compared with the conventional complement-fixation test (CFT) used in Australia. Stored plasma from three Victorian dairy herds with a history of Johne's disease (JD), sera submitted from animals showing clinical signs of JD, and sera from the US National Repository for Paratuberculosis Specimens were used to determine the sensitivity of each test. The EIA detected 48.8% of 43 Australian animals with subclinical JD, while the CFT detected only 12 (21.4%) of 56 subclinically affected cattle. Of 150 subclinically infected US cattle, the EIA detected 47.3% and the CFT detected 52.0%. The EIA detected 59.7% of animals which at the time of sampling were shedding M. paratuberculosis in their feces, but showed no clinical signs of JD, while the CFT detected 57.3%. The EIA correctly identified 88.2% of 136 histologically confirmed clinical cases, and the CFT detected 83.4%. The specificity of each test was determined by testing sera collected at slaughter from animals residing in a known JD-free area of Australia, and from samples from the US National Repository of Paratuberculosis Specimens collected from certified-free-herds in Wisconsin. The EIA was found to have a specificity of 99.8% when 998 Australian animals were used as the test population, and 99.0% when 196 US animals were used. The specificity of the CFT using Australian samples was 96.9% and 95.2% using American samples.

Title Application of multiple diagnostic tests to the diagnosis and profiling of M. paratuberculosis infected herds

Author(s) Andersen PR, Seymour CL, Sockett DC[1], Collins MT[1].

Institution IDEXX Laboratories, Portland, ME 04102 USA. [1]Department of Pathobiology, University of Wisconsin, Madison, WI 53760.

Abstract To obtain a better understanding of disease progression and provide a means of profiling the disease within a herd, three types of diagnostic tests were applied to study herds. Humoral immune response to *M. paratuberculosis* was evaluated using an absorbed antibody ELISA.

Cellular immune response to *M. paratuberculosis* was evaluated by measuring the release of bovine g-IFN from whole blood cell cultures. Cattle shedding *M. paratuberculosis* were identified using either culture or the *M. paratuberculosis* specific DNA probe. Utilization of the two tests to measure immune response gave results which exhibited good sensitivity and specificity and allowed detection of immunologically sensitized animals at both early and late stages of infection. Information from the g-IFN assay can be used in conjunction with the absorbed antibody ELISA and the DNA probe to create a herd profile in less than a week. Monitoring both disease progression and eradication on a herd basis and in individual animals can be performed using a herd profile and repeated testing of the herd.

Title Serodiagnosis of M. paratuberculosis infection using lipoarabinomannan and antigen D in enzyme linked immunosorbent assays: comparison with agar gel immunodiffusion in sheep and after pre-absorption of sera with M. phlei in cattle

Author(s) Sugden EA, Michaelides A, Bosse J.

Institution Agriculture Canada, Animal Diseases Research Institute, NEPEAN, P.O. Box 11300, Station H, Nepean, Ontario, Canada, K2H 8P9.

Abstract This study sought further improvements in the serodiagnosis of paratuberculosis. Comparing paratuberculosis infected sheep with non-infected sheep the agar gel immunodiffusion test for paratuberculosis using antigen D (D-AGID) was superior to enzyme linked immunosorbent assays using lipoarabinomannan antigen (LAM-ELISA) and protein antigen D (D-ELISA), although used in conjunction with D-AGID the LAM-ELISA showed an increase in specificity. For paratuberculosis infected sheep the LAM-ELISA test showed good agreement with D-AGID (kappa = 0.74), but there was less agreement for sheep submitted for routine serodiagnosis for which the infection status was unknown (kappa = 0.19). A large number of LAM-ELISA positive routine sheep sera were not D-AGID positive, and one-third of the D-AGID positive routine sera were missed by LAM-ELISA. Pre-absorption of sera from paratuberculosis-infected and paratuberculosis-free cattle with M. phlei resulted in an increase in LAM-ELISA specificity from 90.7% to 97.3% at a sensitivity of 70.8% relative to LAM-ELISA conducted without pre-absorption.

Title Evaluation of the sensitivity and specificity of diagnostic tests for Johne's disease and other diseases with long incubation or latent periods.

Author(s) Ridge SE, Morgan IR.

Institution Victorian Institute of Animal Science, Attwood, Victoria, 3049, Australia.

Abstract The evaluation of diagnostic tests for Johne's disease in cattle presents special problems because of the pathogenesis of the disease, in particular the long incubation period, and the lack of an efficient diagnostic standard. The development of an antibody response and detectable lesions may only occur many years after an animal is initially infected. The definition of the diseased state in clinically affected cattle is relatively easy since the presence of clinical signs is highly correlated to the existence of gastrointestinal lesions. In animals with subclinical infection, particularly younger animals, the diseased state is less easy to determine. The only way to determine whether an animal is infected may be to retest the animal for an extended period to allow an opportunity for the disease to progress to a stage where a definitive diagnosis can be made. A lengthy period of testing using existing diagnostic tests may therefore be required to determine whether an animal which is positive to a new test is truly infected. In younger animals even necropsy with histopathology and cultural examination, may not detect infected animals. The use of an age correlated sensitivity is suggested as a method of more accurately portraying the efficiency of diagnostic tests for Johne's disease. Similarly, it is difficult to define animals as non-infected when they are derived from populations where Johne's disease is present. Examples of the effects of these factors on the determination of sensitivity and specificity will be presented.

Title Estimating the true prevalence of paratuberculosis from the apparent prevalence when test sensitivity is not constant.

Author(s) Sockett DC, Carr DJ, Collins MT.

Institution School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706 and Wisconsin Department of Agriculture Trade and Consumer Protection, Madison, WI 53705 USA.

Abstract Samples from the National Repository for Paratuberculosis specimens were analyzed to determine if the prevalence of disease affected the sensitivity of the diagnostic tests. Logistic regression indicated that the prevalence of paratuberculosis significantly affected the sensitivity of conventional and radiometric fecal culture, a commercial DNA probe (IDEXX Corporation) and two commercial ELISA tests (Allied Laboratories and Commonwealth Serum Laboratories) (p less than 0.03). The standard complement fixation test (pless than 0.07) and commercial agarose immunodiffusion test (ImmuCell) (p less than 0.10) were not significantly affected. In addition, as the prevalence of paratuberculosis increased, the proportion of animals shedding M. paratuberculosis in their feces also increased (p less than 0.0001). This observed drop in test sensitivity as the prevalence of paratuberculosis declines has important implications for Johne's disease control programs.

Title Use of an absorbed serum ELISA for diagnosis of paratuberculosis in Danish dairy cattle.

Author(s) Bech-Nielsen S[1], Jorgensen JB[2], Ahrens P[2], Feld NC[2].

Institution [1]Dept. of Vet. Prev. Med., College of Vet. Med., The Ohio State Univ., Columbus, OH 43210;[2]National Vet. Serum Lab., P.O. Box 373 DK-1503, Bulowsvej, Copenhagen V.

Abstract This study describes the response of cattle to an enzyme-linked immunosorbent assay (ELISA) for paratuberculosis, using serum from each animal. The results of fecal culture confirmed clinical suspect cases were analyzed in relation to the amount of colonies isolated from the animals on fecal culture (0,+,++,+++,++++ and above). A significant increase in ELISA response in animals with heavy *M. paratuberculosis* shedding (++++ or above) using both unabsorbed and absorbed serum was found, as compared to animals that were fecal culture negative or shedding *M. paratuberculosis* at lower levels (++++), (P less than 0.05). The effects on sensitivity and specificity of using different cut-off points in the different test groups was described, since sera were not discretely segregated into distinct groups of positives and negatives. The specificity of the ELISA in two fecal culture negative herds was 100% at an ELISA cut-off of 0.1 optical density (O.D.) and above for absorbed serum. For unabsorbed serum the specificity was 62.9% of a similar cut-off value. Similarly the specificity of a fecal culture negative, serologic positive herd increased from 37.5 to 72.2 at an ELISA cut-off value of 0.1-0.2 (O.D.) using absorbed versus unabsorbed serum, and from 75.0 to 94.4 at an ELISA cut-off value of 0.2-0.3.

Title Case definition of paratuberculosis for diagnostic test sensitivity and specificity determination

Author(s) Sockett DC, Carr DJ, Collins MT.

Institution School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706 and Wisconsin Department of Agriculture Trade and Consumer Protection, Madison, WI 53705 USA.

Abstract It is important when evaluating diagnostic test accuracy to include the entire spectrum of disease which is normally found in the population being tested. For Johne's disease this should include disease-free populations of animals for specificity analysis and infected populations of animals, composed of both those shedding *M. paratuberculosis* in their feces and those not shedding, for sensitivity analysis. Data from the National Repository for Paratuberculosis specimens will

be presented to illustrate how the choice of samples for test sensitivity and specificity analysis can influence test accuracy determination. For example, if sensitivity analysis was done using only culture-positive cows and specificity analysis was done on animals in the same herd but having a minimum of three negative fecal cultures over a 24 month period, the sensitivity and specificity of the standard complement fixation (CF) test used in the United States (greater than or equal to 1:8 = positive) would have been 54.6% and 89.2%, respectively. In reality, the CF test had a sensitivity and specificity of 38.4% and 99.0%, respectively, based on biopsy or necropsy and isolation of *M. paratuberculosis* from tissue specimens. Similar findings were found for all the diagnostic tests examined and points out the need to critically evaluate the populations of animals used to determine the sensitivity and specificity of a diagnostic test. If this is not done, erroneous conclusions will be made about the accuracy of diagnostic tests for paratuberculosis, particularly for tests not based on organism detection.

Title Evaluation of milk ELISA testing for detection of dairy cows infected with M. paratuberculosis

Author(s) Sweeney RW, Whitlock RH, Buckely C.

Institution Department of Clinical Studies, New Bolton Center, Univ. of Penn. School of Veterinary Medicine, Kennett Sq., PA 19348, USA.

Abstract Milk and serum samples were collected from 16 cows naturally infected with *M. paratuberculosis* (detected by fecal culture; centrifugation method) and 9 uninfected cows (based on at least 3 negative annual fecal cultures). Samples were subjected to ELISA testing using 3 antigens: L-arbinomannan (LAM), protoplasmic (PPA), and protoplasmic following adsorpiton of *M. phlei* (Commonwealth Serum Laboratories [CSL]). Serum samples were tested with the LAM antigen within 24 hours of collection (fresh), and after being frozen (-70°C) for 1 year; only frozen samples were tested with PPA and CSL antigens. Fresh and frozen milk samples were evaluate with and without chemical preservative (Bronopal, brominated hydrocarbon) with the LAM antigen; only frozen samples were evaluated with PPA and CSL antigens. Milk ELISA scores were significantly correlated with serum ELISA scores (P less than 0.0001) for all 3 antigens, with r values ranging from 0.79 to 0.92. Repeated measures analysis of variance revealed no signficant difference between fresh vs. frozen milk or serum samples, nor was there any significant difference for preserved vs. unpreserved milk samples. Milk and serum ELISA scores were significantly higher for fecal culture-positive cows when compared with fecal culture-negative cows. However, there was considerable overlap in serum and milk ELISA scores for culture-positive and culture-negative cows. Apparently, freezing of serum and milk samples for 1 year at -70°C, and addition of chemical preservative to milk samples does not significantly affect the outcome of ELISA testing.

Title Comparative evaluation of conventional double incubation culture methods for the isolation of M. paratuberculosis from bovine fecal samples

Author(s) Singh SN, Maddux RL, Kadel WL.

Institution Murray State University, Breathitt Veterinary Center, Hopkinsville, Kentucky 42240 USA.

Abstract This investigation was conducted to evaluate the efficiency of conventional old method with that of the new method (two step germination) in our diagnositic laboratory using 5.0 g samples for fecal culture. Using the old method, out of a total of 417 suspected clinical fecal samples cultured, 170 (40.8%) were positive with a contamination rate of 4.96%. During the same period, out of 625 herd samples, 17 (2.7%) were found positive with a 7.2% contamination rate. Using the two step germination procedure, out of a total of 335 suspected clinical fecal samples, 135 (40.29%) yielded positive culture with a contamination rate of 2.08%. Whereas, out of 450 herd samples, 10 (2.22%) were found positive. Furthermore, the same 69 clinical fecal samples were compared in parallel cultures using both methods, of which 26 (37.68%) and 27 (39.1%) were positive by the old and new methods, respectively, indicating a higher sensitivity of the new method. In addition, the new method was considerably superior in reducing the rate of

culture contamination.

Title Culture techniques and media constituents for the isolation of M. paratuberculosis from bovine fecal samples.

Author(s) Whitlock RH[1], Rosenberger AE[1], Sweeney RW[1], Hutchinson LJ[2].

Institution [1]New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348, USA and [2]Dept. of Veterinary Science, Penn State University, University Park, PA 16802, USA.

Abstract A variety of techniques and concentrations of media constitutents in Herrold's egg yolk media were evaluated to assess their influence on the recovery of *M. paratuberculosis* from bovine fecal samples. Sodium pyruvate (4.1 gms/L) enhanced both growth and recovery of *M. paratuberculosis* compared to media without pyruvate. An increase of 20% egg yolk per liter of media improved *M. paratuberculosis* recovery. Centrifugation at more than 900 G for thirty minutes did not seem to increase the recovery rate. Increasing the sample size from one (1) to two (2) grams had a major influence, but further increases in sample size had only a minor influence. A 50% increase in Mycobactin-J did not increase recovery while a reduction (50%) in malachite green increased bacterial contamination while decreasing recovery of *M. paratuberculosis*. Fecal samples mixed with a commercial lubricant did not reduce recovery. Storage of fecal samples at room temperature, 4°C or -20°C did not reduce recovery of *M. paratuberculosis* when stored in these conditions for up to one week.

Title Gastrointestinal transit of M. paratuberculosis in cattle following oral inoculation.

Author(s) Sweeney RW, Whitlock RH, Herr SA, Rosenberger EA.

Institution Department of Clincal Studies, New Bolton Center, Univ. of Pennsylvania School of Veterinary Medicine, Kennett Sq. PA 19348 USA.

Abstract The objective of this study was to determine whether orally ingested *M. paratuberculosis* could cause a "false" positive fecal culture result in uninfected cattle. Six uninfected Guernsey heifers (age 2 years) were given feces from a heavily infected cow with clinical signs of paratuberculosis. Each pair received one of three doses (2 ml feces/kg body weight, 0.5 ml/kg, or 0.12 ml/kg) by orogastric tube. Feces were collected three times daily and cultured for *M. paratuberculosis* on Herrold's egg yolk medium using the centrifugation technique. Intradermal johnin tests were performed prior to and 14 days after the dosing. Serum was collected for ELISA testing (LAM antigen) prior to and 7 and 14 days after dosing. All heifers had detectable *M. paratuberculosis* organisms in feces within 24 hours of treatment, and cultures remained positive for up to 6 days. The concentration of *M. paratuberculosis* organisms in the feces was approximately proportional to the dosage of infected feces given. Results of intradermal johnin testing and serum ELISA testing remained negative throughout the experiment. The heifers were given the same dose a second time, 14 days after the first dose, with similar results. Two weeks following the second dose, (28 days after the first dose), the heifers were slaughtered and ileum and mesenteric lymph nodes collected for mycobacterial culturing and histopathology. All lymph nodes were culture-negative but ileum samples were culture-positive. Histopathology revealed mild eosinophilic inflammation in the mesenteric lymph nodes and no acid fast staining organisms. Ingestion of feces from an infected cow can apparently result in a positive fecal culture test result in uninfected cattle.

Title Evaluation of three diagnostic DNA probes tests for Johne's disease on two dairy farms in the Netherlands

Author(s) van der Giessen J[1], Haring R[1], Eger T[2], Haagsma J[2], van der Zeijst B[1].

Institution [1] Institute of Infectious Diseases and Immunology, University of Utrecht and [2]Central Veterinary Institute, Lelystad, The Netherlands.

Abstract A DNA probe specific for the 16S ribosomal gene of *M. paratuberculosis* was developed. This probe was used in a polymerase chain reaction (PCR) based assay for detection of *M. paratuberculosis* in fecal material of infected cattle. Comparison with the results of culture show that there were no false positive results. However, the sensitivity was considerably lower. A field evaluation was carried out on two dairy herds with a long history of Johne's disease. Two other DNA probe tests were also included in this evaluation. One of these tests used the same fecal preparation method, but the PCR primers were derived from the *M. paratuberculosis* specific IS900 insertion element. The third test was the commercially available IDEXX *M. paratuberculosis* DNA probe test. The sensitivity of the 3 DNA probe tests ranged from 13-234% compared to culture results, The specificity of these tests under field conditions was 100%. We conclude that DNA probe tests give rapid and specific results. More research has to be done to increase the sensitivity.

Title Comparison of IDEXX DNA probe test and three cultivation methods for detection of M. paratuberculosis in bovine feces.

Author(s) Whipple DL[1], Kapke PA[1], Andersen PR[2].

Institution [1] USDA, ARS, National Animal Disease Center, Ames, IA 50010 USA and [2] IDEXX Corporation, Portland, ME 04101 USA.

Abstract Diagnosis of paratuberculosis using the IDEXX DNA probe test and three methods for cultivation of *M. paratuberculosis* from fecal specimens were compared. Twenty-one of 170 fecal specimens were DNA probe test positive, whereas 35 specimens were culture positive by one or more of the methods evaluated. Four specimens were DNA probe positive but culture negative. The probe test detected *M. paratuberculosis* DNA in 62.9% of specimens positive by a sedimentation method, 56.6% of those positive by a centrifugation method, and 65.4% of those postive by the Cornell culture method. Generally, the probe test was positive for cattle shedding at least 10^4 *M. paratuberculosis* organisms per gram of feces. It was possible to identify cattle shedding the greatest number of organisms in 3 days using the probe test compared with a minimum of 6 weeks required for positive culture results. After 12 weeks incubation, the centrifugation method resulted in the most isolations of *M. paratuberculosis* and the most contamination of culture medium. Contamination was best controlled using the Cornell culture method. The sedimentation method was the least time-consuming and yielded results similar to the other two methods.

Title Serological, microscopic, cultural and pathological findings from 135 sheep originating from a paratuberculous flock in South Africa.

Author(s) Huchzermeyer HF[1], Bastianello SS[2].

Institution [1]Section of Bacteriology, [2]Section of Pathology, Veterinary Research Institute, Onderstepoort 0110, Republic of South Africa.

Abstract Paratuberculosis was diagnosed in 1988 in South Africa on histopathological examination of an emaciated sheep which originated from a flock of sheep at an agricultural research station in South Africa. This paper deals with the findings from 135 slaughtered sheep from the above paratuberculous flock. The sheep had not been selected as suspected clinical cases of paratuberculosis. The sheep were examined via the agar gel immuno-diffusion (AGID) test and complement fixation test (CFT), microscopic examination of fecal and intestinal smears, culture and histopathological examination, predominantly of the ileum. The AGID test was strongly positive in 3.1% and weakly positive in 13.9% of the sera. The CFT was positive in 2.3% of the sera. The infection rate by microscopic examination of smears was 2% for feces, 11.1% for the ileum and 10.3% for the caecum. *M. paratuberculosis* has so far been cultured from 4 cases. Histopathological evidence of paratuberculosis was found in 11% of cases examined. There seems to be a poor correlation between the results obtained by the above methods. This demonstrates the necessity of using several available methods in order to attain quantifiable results with reasonable accuracy.

Title Serodiagnosis of paratuberculosis in sheep using agar gel immunodiffusion.

Author(s) Shulaw WP, Bech-Nielsen S, Rings DM, Getzy DM, Woodruff TS.

Institution Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, Ohio 43210, USA.

Abstract An agar gel immunodiffusion test (AGID) utilizing a commercially available antigen, was used to detect *M. paratuberculosis*-infected animals in a field study. Over 2000 serum samples obtained from 5 infected and 4 presumed-uninfected sheep flocks were tested over a 5-year period. A total of 35 AGID-positive sheep were identified and 27 of these were available for necropsy. Infection was confirmed in all necropsied sheep by histopathology, acid-fast staining of ileal mucosal smears, and/or bacterial culture. Histopathology was a reliable method of confirming paratuberculosis and demonstrated a wide spectrum of lesions. Isolation of M. paratuberculosis by culture of feces and tissues of infected animals was successful in only three cases. The AGID was useful in identifying infected sheep with weight loss. In those animals which were repeatedly tested, AGID appeared to identify most infected animals late in the course of disease progression, however, ten animals were in normal body condition when necropsied or lost to follow-up (one animal). Exposure of animals to or infection with Corynebacterium pseudotuberculosis did not appear to cause false positive reactions in the AGID. It was concluded that AGID with the commercially available antigen may be useful to identify *M. paratuberculosis*-infected sheep with weight loss, and that it may be useful in flock testing programs.

Title Comparison of the ELISA, AGID and C.F. tests for diagnosis of caprine and ovine paratuberculosis

Author(s) Dimareli-Malli Z[1], Sarris K[2], Xenos G[1], Papadopoulos G[1].

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Abstract The comparitive results of 55 and 81 serum samples from 8 and 10 flocks of goats and sheep, respectively, examined with ELISA, AGID and CFT are described. The 40 sheep sera originated from a research institute free of paratuberculosis, while all others were from well known infected flocks with paratuberculosis. The three serological methods were evaluated according

to the microscopic examination and culture of fecal samples. In goats, the sensitivity and specificity of the ELISA test was 86.95% and 90.62%, the AGID was 65.21% and 84.37% and the CFT was 39.13% and 75%, respectively. In sheep the sensitivity and specificity of the ELISA test was 83.33% and 86.95%, the AGID was 61.11% and 82.6% and the CFT 55.55% and 82.60%, respectively. The present study indicates that the ELISA test is more sensitive and more specific than AGID and CFT for diagnosis of caprine and ovine paratuberculosis.

Title Prevalence of paratuberculosis in infected goat flocks and comparison of different methods of diagnosis.

Author(s) García Marín JF, Chávez Gris G, Adúriz JJ[1], Pérez V, Juste RA[1], Badiola JJ.

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Abstract Pathological studies of iliocecal valve, ileum and jejunum from forty two goats randomly selected from two paratuberculosis infected flocks were performed. In twenty nine goats, lesion of paratuberculosis were observed. Ten of them had lesions in the Peyer's patches only. Cultures from the same samples were made in Lowenstein-Jensen medium without piruvate and positive results were obtained in twenty one animals, all of them with paratuberculosis lesions. With serum samples, the AGID and ELISA techniques were applied by using PPA-3 antigen. The AGID test had 55.1% sensitivity and 84.6% specificity; ELISA had 65.5%, 84.0% sensititivty and specificity, respectively, using the pathological results as a reference. The ELISA test detected 100% of the animals with severe lesions, 64% with less important lesions and 50% with lesions located in Peyer's patches only. The high prevalence of infection (69%) in paratuberculosis flocks of goats, with poor sensitivity of serological diagnosis tests, which detected animals with severe lesions, must be addressed.

Title The diagnosis of Johne's disease in farmed deer

Author(s) de Lisle GW, Wards BJ, Collins DM.

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Abstract During the last 20 years deer farming has developed into a major pastoral industry in New Zealand. The national deer herd consists of approximately 1,000,000 animals. Johne's disease in farmed deer is rare and there have been only 16 bacteriologically confirmed cases in the last 5 years. This is in marked contrast to the high level of infection in cattle and sheep in New Zealand. Half of the 16 cases were identified at routine meat inspection of clinically normal deer. Gross lesions of Johne's disease are often caseous and similar to those caused by other members of the *Mycobacterium avium* complex or *Mycobacterium bovis*. Characterization of cervine strains of *Mycobacterium paratuberculosis* by restriction endonuclease analysis and DNA hybridization revealed two types, one identical to those found in New Zealand sheep and the other to those from cattle. Clinical Johne's disease in deer occurs very rarely but has often been observed in animals as young as 12 months of age. It appears that rare individuals are very susceptible to mycobacterial infections, including Johne's disease, while the majority of deer have a significant degree of resistance to infection with *M. paratuberculosis*.

Title Prevalence and type of paratuberculous lesions in sheep and their relation with the diagnosis by AGID test

Author(s) García Marín JF, Pérez V, Badiola JJ.

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Abstract Culled sheep, 44 in total, from two sheep flocks with losses due to paratuberculosis were studied. In these animals, pathological studies in samples from the ileocecal valve and ileum (HE and ZN stains) were carried out. Paratuberculous lesions were observed in 26 animals. The lesions were classified as follows: Type I (Focal lesions, with the granulomas located only in the Peyer's patches); Type II (Granulomatous lesions mainly located in Peyer's patches and with invasion of related mucosa); Type III (Diffuse granulomatous lesion involving mucosa nonrelated with Peyer's patches). A total of 14 animals had Type I lesion; 4 had Type II and 8 presented Type III lesion. AGID test were performed on all animals of the flocks, including the culled sheep; 3% of them were serologically positive. Among the culled sheep, 18% were serologically positive (7 out of the 39 tested), 5 of them showed lesions Type III, and 2 lesions Type II. The sensitivity of the AGID test was 27% with 100% of specificity. The low percentage of infected sheep detected by AGID, and the high prevalence of the infection in the flocks with the presence of seronegative sheep with Type I lesion must be addressed.

Title PCR detection of Mycobacterium paratuberculosis in Crohn's Disease Tissue DNA extracts

Author(s) Sanderson JD, Moss MT, Tizard MLV, Hermon-Taylor J.

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Abstract DNA was extracted enzymatically from full-thickness intestinal resection specimens from 40 patients with Crohn's Disease (CD) and 40 cancer or diverticular disease controls. 5' IS900 PCR was performed using primers shown to be capable of detecting a single *M. paratuberculosis* genome. Under optimal conditions 300 bacilli could be detected in 1g of tissue. *M. paratuberculosis* DNA was detected in 26 of 40 (65%) CD tissues and in 5 of 40 (12.5%) control tissues. Stringent precautions were taken to exclude artefact due to laboratory contamination. The presence of *M. paratuberculosis* in a small proportion of control tissues is consistent with a previously unrecognized environmental prevalence. Its presence in a majority of CD tissues is consistent with a role for *M. paratb* in the pathogenesis of this disease.

Title Identification by PCR of M. paratuberculosis in long term cultures from Crohn's disease tissue

Author(s) Moss M, Sanderson J, Tizard M, Hermon-Taylor J, El-Zaatari F, Markesich D, Graham D.

Institution Department of Surgery, St. George's Hospital Medical School, UK. Digestive Disease Section, VAMC and Baylor College of Medicine, Houston, Texas.

Abstract Isolation of *M. paratuberculosis* from a small number of Crohn's disease (CD) patients has led to renewed interest in the potential role of this organism in the pathogenesis of CD. *M. paratuberculosis* grows extremely slowly in vitro and yields are invariably low. PCR, however, is ideal for the detection of small numbers of bacilli. Centrifugal pellets from cultures of intestinal tissue from 18 CD patients and 11 non-CD controls were tested for the presence of *M. paratuberculosis* DNA using a PCR assay based on the 5' region of IS900. In 6 of the 18 CD's and 1 of the 11 controls *M. paratuberculosis* was positively identified. The intensity of the PCR signal in the positive CD samples was consistent with an abundance of M. paratuberculosis approximating 5-30 genomes per culture pellet. The results are consistent with the presence of *M. paratuberculosis* in intestinal tissue in CD, in a form which hardly replicates in culture.

Title Antibodies against M. paratuberculosis and professional activity.

Author(s) Juste RA, Saez e Ocariz C, Beltran de Heredia F, Agud J, Portu J, Aldamiz-Etxebarria M.

Institution SIMA, 48016 Derio (Bizkaia) SPAIN.

Abstract As evidence on infection by *M. paratuberculosis* in human beings is increasing, we tested the level of antibodies in a sample of slaughterhouse or related activities workers compared with a control one of administrative workers. The total number of sera were 1312 in the immunodiffusion (ID) test and 1277 in the ELISA. Both methods were carried out with a protoplasmatic antigen following the instructions of the supplier (Allied Laboratories,Inc.). Each sample was submitted to the ELISA in duplicate, using either an anti-IgM or an anti-IgG conjugate. The results were expressed as the ratio of the optical density (OD) of each serum to the OD of a normal reference serum included in each plate. In the ID test 7 sera gave a positive reaction, 2 belonging to the control sample and 5 from the slaughterhouses sample, resulting in 0.53% of seroreactivity in the whole sample. However, 4 of the positives corresponded to a single slaughterhouse, in which the percentage of positives reached 1.52%. In the ELISA, the comparison with the control group indicated a signficiantly higher IgM index in 7 places. For the IgG, out of a total of 6 significant differences, only 2 places showed higher indexes, while the other 4 (poultry slaughterhouses) had lower ones. Five places showed significant differences in both Ig's. In regards to the type of activity, the highest indices corresponded to tripe butchers, veterinarians, slaughterers and transporters for IgM, while for IgG the highest indexes were

observed for tripe butchers and veterinarians and the lowest for transporters. Our results showed a reactivity of human beings against antigens of the avium-paratuberculosis group. Although it could be explained by just an environmental contact, the fact that two clear patterns seem to arise from the IgM/IgG reactivity, with the cases of increase in both mostly related to contact with ruminants, leads to suspect some specific relationship with paratuberculosis that seems to deserve a more thorough study.

Title Variability of the presence of antibodies against a 65 Kd antigen in sera from goats infected with M. paratuberculosis.

Author(s) Crespo M, Moreno A, Molina A, Llanes D.

Institution Departamento de Genetica. Instituto de Zootecnia. Facultad de Veterinaria. Cordoba, Spain.

Abstract In order to study the different antigenic components recognized by sera from paratuberculosis goats, sera from a) goats considered pathologically infected by *M. paratuberculosis* (10); b) goats (4) and rabbits (4) hyperimmunized with PPA-3* and a goat strain of M. paratuberculosis and c) ELISA positive goats (10), were analyzed by western-blotting using PPA-3 as antigen. More than ten different major antigenic components were recognized by the sera in all the goats analyzed. A specially concentrated antigenic cluster appeared between 45-35 Kd. This cluster is almost invariable in all positive goat sera studied, and it was not present in healthy or negative animals. A specific antigen of around 65 Kd was present in only some sera from pathologically infected goats. This antigen was not removed by absorption with *M. phlei* and was not identified in the sera from rabbits nor goats immunized with PPA-3, although it was present in goats immunized by *M. paratuberculosis* and in some sera from ELISA positive animals. This antigen was recognized by 7 of 28 goats studied. This antigen could be related to the 65 Kd antigen of other mycobacteria and its presence in only some animals may be the result of an autoimmune disease produced in some animals infected with *M. paratuberculosis.*

Title Identification and characterization of immunogenic proteins of M. paratuberculosis

Author(s) Stevenson K, Inglis NF, Rae B, Burrells C, Donachie W, Sharp JM.

Institution Moredun Research Institute, Edinburgh, Scotland, UK.

Abstract A research program has been undertaken to investigate the pathogenesis of *M. paratuberculosis* and *M avium* in deer and to determine those antigens that elicit specific humoral and cellular immune responses. A number of recombinent clones expressing immunogenic antigens have been isolated from a gt11 genomic library of *M. paratuberculosis* using sera from clinically infected or experimentally inoculated animals. The recombinent clones have been lysogenized in Escherichia coli Y1089 and the *M. paratuberculosis* antigens encoded by the clones have been identified using antibody eluted from fusion protein bands on Western blots. The recombinant clones contain DNA sequences coding for antigens of approximately 33 kd and 28 kd and for the 70 kd heat shock protein. In addition, a soluble protein of 30 kd has been purified from *M. paratuberculosis* cell lysates which elicit strong humoral and cellular immune responses in infected animals. The N-terminal amino acid sequence of this protein has been shown to share 90% homology with those of the mature 30 kd alpha-secretory antigens of *M. bovis* and *M. kansasii* and also with a 32 kd secretory antigen of *M. tuberculosis.*

Title Experimental paratuberculosis in severe combined immunodeficient/biege mice.

Author(s) Mutwiri GK, Butler DG[1], Rosendal S, Percy D[2].

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Abstract Severe combined immunodeficient/beige (Scid/bg) mice were infected intraperitoneally (IP) with a single injection of 1 x 10^5 *M. paratuberculosis* (MPTB) organisms. There was histologic evidence of infection within one month of inoculation. The earliest lesions consisted mainly of foci of mononuclear cells with and without acid-fast bacteria. These lesions which gradually enlarged and coalesced developed in a time-dependent fashion, appearing first in the liver, then the spleen and finally in the intestines. No lesions/acid-fast organisms were ever seen in the heart, kidney and lungs of test or control mice. By 12 weeks post-infection, mice

developed significant weight loss compared to their uninfected controls, and by 22 weeks becmae emaciated, mimicking clinical paratuberculosis in ruminants. Tubour necrosis factor-alpha was not detectable in the plasma of infected mice as determined by a double sandwich ELISA. Our results show that Scid/bg mice can be easily and consistently infected I.P. (58 out of 58) with relatively few MPTB organisms and consistently develop cachexia. We propose the Scid/bg mouse as a useful model for the study of immunopathogenesis, cachexia and therapy in MPTB infections.

Title IS900, the p43 protein and M. paratuberculosis

Author(s) Tizard M, Moss M, Sanderson J, Hermon-Taylor J.

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Abstract The novel insertion sequence IS900 is unique to *M. paratuberculosis* and has been successfully used as a specific and sensitive DNA probe. IS900 protein expression was studied. As predicted from the nucleotide sequence, a single protein, p43, was produced. This protein was readily over expressed in recombinant *E. coli*.. A synthetic peptide, from the predicted amino acid sequence, was used to raise a p43 mono-specific antiserum in rabbits. This reagent identified p43 in Western blots of protein extracts from *M. paratuberculosis* and recombinant *E. coli* but not extracts of *M. avium*. The level of p43 is unusually high for the transposase of an IS element. This has important implications for the relationship between IS900 and *M. paratuberculosis*. p43 is a hydrophilic protein and is potentially antigenic. The protein has been extracted from recombinant bacterial lysate by affinity chromatography to 95% purity. This will provide a defined antigen to test antibody and cell mediated immune response to *M. paratuberculosis* and is a potential diagnostic reagent.

Title Screening of a M. paratuberculosis expression library with polyclonal antiserum and amplified DNA probes to identify species-specific immunodominant antigens.

Author(s) El-Zaatari FAK, Engstrand L, Markesich DC, Graham DY.

Institution Inflammation Bowel Disease Lab, VAMC and Baylor College of Medicine, Houston, TX 77030 USA.

Abstract The eradication of the economically important disease, Johne's disease, has been hampered by the lack of simple accurate diagnostic tests. We have begun to dissect the antigenic structure of *M. paratuberculosis* to identify the species-specific antigen(s) required for development of a sensitive and specific diagnostic test and to understand the role of individual proteins in the pathogenesis of Johne's disease and possible Crohn's disease. An *M. paratuberculosis* (strain linda) expression library was constructed in a phagmid vector and screened with adsorbed hyperimmune rabbit anti-*M. paratuberculosis* serum and pooled polymerase chain reaction (PCR)-DNA product probes representing 65K and 32K-encoding genes and the IS900 of *M. paratuberculosis*. The derived recombinants contained 3 to 5 mycobacterial genome equivalents; 60% of recombinants contained inserts with the mean size of 2.8Kb. Three recombinants from 116 to 65 independent clones reacted with antibodies and pooled PCR-DNA probes, respectively, reacted with both antisera and PCR-DNA product probes. 25 of 29 clones selected by the pooled PCR probes reacted with the IS900 PCR-probe; 4 clones including the expressed one hybridized with 32K and 65K PCR DNA probes. Because 65K and 32K antigens are known to be major stimulants of cellular and humoral immunity against mycobacteria, our 4 putative species-specific clones are under investigation to identify species-specific epitope(s) useful in the development of rapid diagnostic tests as well as in the investigation of their role in pathogenesis of disease.

Title The role of T and NonT/nonB (N) lymphocytes in the immune response to Mycobacteria

Author(s) Davis WC[1], Chiodini RJ[2], Monaghan M[3].

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Abstract Studies undertaken to detail the composition of the bovine immune system have shown it is composed of three partially overlapping populations of lymphocytes: B cells defined by the expression of sIgM and lineage specific membrane molecules, T cells defined by the expression of the alpha-beta TCR, BoCD2, -CD3, -CD5, -CD6 in association with -CD4 or -CD8, and nonT/nonB (N) cells defined by the expression of an unique molecule N12, -CD3, and -CD5. Analysis of the N12+ population has shown it is composed of two subpopulations: 1) a large population composed of multiple subsets that express the gamma delta TCR, workshop cluster 1 (SC1), and lineage specific molecules, and 2) a small population composed of subsets that express -CD2 and -CD6 alone or in association with -CD8 or -CD8 and the gamma delta TCR. Preliminary studies suggest gamma delta + N lymphocytes may play a significant role in the regulation and expression of T cell responses to *M. bovis* and *M. paratuberculosis* in ruminants.

Title The cellular immunology of bovine paratuberculosis: The predominant response is mediated by CD8+ and N cells which suppress CD4+ activity.

Author(s) Chiodini RJ, Davis WC.

Institution Brown University, Providence, Rhode Island and Washington State University, Pullman, Washington.

Abstract Using a blastogenesis assay of peripheral blood mononuclear cells depleted of specific lymphocyte subsets, various subset add-back experiments, and examination of T cell lines from an animal immunized with *M. paratuberculosis*, we examined the functional activity of T cell subsets in response to infection. Initial response to *M. paratuberculosis* antigens was mediated by BoCD4+ T helper lymphocytes reacting with cell-wall antigens during the first 2-3 months. Cellular concentrations of BoCD4+ lymphocytes then rapidly declined and anergy developed. Responsiveness during this 70 day period could not be restored. A second proliferative phase was shown to be mediated by BoCD8+ and N cells reacting to soluble antigens of *M. paratuberculosis*. N and CD8+ cell populations accounted for the total proliferative response of peripheral blood cells. By a series of add-back experiments and examination of N2+ cell lines, it was concluded that antigen-specific BoCD8+ and N populations respond to M. paratuberculosis antigens by a BoCD4+-independent mechanism. Proliferation of BoCD8+ and N cells were also independent of each other. The BoCD8+ cell population exerted a moderate suppressive effect on both specific and nonspecific responses, but no antigen-specific suppression by the BoCD8+ population could be demonstrated. The N population, however, exhibited an antigen-specific suppression of BoCD4+ cells. Add-back experiments illustrated that the BoCD4+ population contained *M. paratuberculosis*-specific reactive cells which failed to proliferate as a result of an antigen-specific suppression by the N population. It was concluded that the inability of effector cell populations to prevent intracellular proliferation of *M. paratuberculosis* was due to suppression of the T helper lymphocyte population required for macrophage activation.

Title The immunopathogenesis of ruminant paratuberculosis.

Author(s) Snider TG III[1], Olcott BM, Kreeger JM, Hines II ME, Turnquist SE, Vance TL, Farrar RG.

Institution [1]Dept. of Veterinary Pathology, School of Veterinary Medicine, Louisiana State University and A&M College, Baton Rouge, LA 70803.

Abstract Data from clinical cases, abattoir and serologic surveys have documented a substantial prevalence of paratuberculosis in Louisiana. Studies of cattle with chronic ruminant paratuberculosis have utilized histologic techniques to support in vivo and in vitro assays of immunological function. Dermal hypersensitivity, lymphocyte blastogenesis, Interleukin-1 and IL-2 assays have indicated the presence of various immunological perturbations during the chronic disease. Peripheral blood macrophages spontaneously release Interleukin-1 and this offers a possible explanation for the absence of concurrent or secondary disease. Studies of *M. paratuberculosis* strain 18, yielded the isolation of 2 or 3 different components which inhibited killing of Candida albicans by LPS-PMA activated adherent peripheral blood macrophages. The in vivo activity of these factors has been supported by growth of viable bacilli in activated bovine macrophage cultures. Murine infections at 8 weeks yielded viable organisms with evidence of ineffective lymphoid stimulation. These results suggest a mechanisms for the occurrence of regional granulomatous disease without systemic immunosuppression and for the ability of infected cattle to resist secondary diseases.

Title Preliminary evidence for the importance of gamma delta T cells in the immunity to ruminant paratuberculosis

Author(s) Veazey RS[1], Snider TG III.

Institution [1]Dept. of Veterinary Pathology, School of Veterinary Medicine, Louisiana State University and A&M College, Baton Rouge, Louisiana 70803.

Abstract Since their discovery in 1984, the function of the gamma delta subset of T cells has been the subject of much debate. The fact that large numbers of these cells reside in the epidermis and the intestinal epithelium suggests that they play an important role in the surveillance of epithelial barriers to invading organisms. Recent studies have shown that these cells are particularly prominent in the intestinal mucosa of young ruminants. In vitro studies have shown that this subset of cells selectively proliferate in response to antigens from both *M. tuberculosis* and *M. bovis*. These findings suggest that this subset of cells may play an important role in the pathogenesis of ruminant paratuberculosis.

Title Bovine T helper (BoCD4+) lymphocytes recognize a limited antigenic repertoir of M. paratuberculosis

Author(s) Chiodini RJ, Davis WC[1], Brennan PJ[2].

Institution Dept. of Medicine, Brown Univ and the Rhode Island Hospital, Providence, RI, [1]Dept Vet Microbiol Pathol, Washington State University, Pullman, WA, and [2]Dept Microbiology, Colorado State University, Fort Collins, CO.

Abstract It has recently been demonstrated that cellular responses in cattle chronically infected with *M. paratuberculosis* are regulated by nonT/nonB (N) lymphocytes of the N2+ subpopulation which suppress antigen specific T helper (BoCD4+) lymphocytes. The inactivation of the helper cell population required for macrophage activation may be the underlying mechanism by which *M. paratuberculosis* is able to replicate. It was also shown that immunity could be expressed by the emergence of a new BoCD4+ population in conjunction with BoCD8+ contrasuppressor cells. Examination of BoCD4+ PBMC and BoCD4+ cell lines illustrated that cellular reactivity was directed, almost exclusively, to a cell wall associated antigen fragment of *M. paratuberculosis.* Helper reactivity to this antigen is MHC-restricted, requiring the participation of adherent cells to proliferate. The antigen is approximately 700,000 to 1x106 molecular weight and can be found in small quantities within some preparations of Johnin PPD. BoCD4+ cell lines reactive against this antigen fail to respond to *M. avium* or avian PPD (including strain 18). This antigen may have application in the identification of resistant animals, development of a specific subunit vaccine, and immunotherapy of infected animals.

Title In vivo and in vitro cell-mediated immune responses in paratuberculous cattle after sensitization with homologous and heterologous antigens.

Author(s) Kreeger JM, Snider TG III.

Institution Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO 65211 and Department of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 USA.

Abstract Lymphocyte blastogenesis, monocyte migration inhibition and cutaneous hypersensitivity were measured in cattle with chronic paratuberculosis before and after sensitization with *M. bovis*, *M. paratuberculosis*, and keyhole limpet hemocyanin in Freund's incomplete adjuvant. The greatest in vitro responses were seen with *M. bovis* antigen and no differences were detected pre- and post-sensitization with these assays. Pre-sensitization skin testing with *M. bovis* PPD and johnin was variable with a significant decrease in cutaneous responses after sensitization with the mycobacterial antigens. In vitro and in vivo reactivity to keyhole limpet hemocyanin was negligible in infected cattle both before and after sensitization with KLH. These results indicate that cattle with paratuberculosis respond suboptimally to heterologous antigen sensitization and are prone to desensitization when homologous antigens are administered.

Title Mycobacterial glycolipid fractions inhibit activated macrophages.

Author(s) Hines II ME, Snider TG III.

Institution University of Miami, School of Medicine, Department of Comparative Pathology, Miami, FL 33101 and Department of Veterinary Pathology, Louisiana State University, Baton Rouge, Louisiana 70803.

Abstract Glycolipid fractions that inhibit the killing of Candida albicans (CA) by activated bovine peripheral blood derived macrophages (Mo) were derived from *M. paratuberculosis* strain 18 (*M avium* serovar 2). Active fractions were derived utilizing the Matrix Solid Phase Dispersion technique, which is a novel method of lysis and partial fractionation of components of bacterial cells. Further fractionation of active fractions was achieved using Concanavalin A affinity chromatography and centrifugal filtration. As many as three different fractions derived from two *M. paratuberculosis* extracts that exhibited marked inhibition of Mo killing ability have been isolated and partially characterized. Two active fractions demonstrated characteristics typical of glycolipids and a third active fraction had characteristics compatible with a peptidoglycolipid.

Title Study of the entrance of M. paratuberculosis in the lambs intestinal mucosa using immunohistochemical methods for antigen detection.

Author(s) García Marín JF, Benazzi S, Pérez V, Badiola JJ.

Institution Facultad Veterinaria. 50013 Zaragoza Spain.

Abstract To study the entrance of *M. paratuberculosis* in the intestinal mucosa of sheep, ten one month old lambs were orally infected with 5 x 10^{10} *M. paratuberculosis* , ovine strain. In groups of two, they were killed at the following intervals: 12 hours p.i., 36 h.p.i., 3, 7 and 14 days p.i. We used Z-N stain and immunohistochemical technique of ABC-Peroxidase, using a primary antibody against *M. paratuberculosis* antigens, in samples from the ileocecal valve, ileal Peyer's patch, nine jejunal Peyer's patches and jejunal mucosa located between Peyer's patches. The samples were fixed in formalin and embedded in parafin. Positive results using ABC-Peroxidase technique were found at 3, 7 and 14 d.p.i., the maximum positivity detected at 7 d.p.i.. The antigens of *M. paratuberculosis* were mainly observed in the M cells of the dome of Peyer's patches. Macrophages subepithelially located in the domes were sporadically positive, and a few positive cells were observed in the epithelium of those villi close to the domes, with high positivity. According to these results, the antigens of *M. paratuberculosis* detected by immunohistochemical methods but negative by Z-N stain, suggest the presence of L forms, during the initial phases of infection, which would be located in the cytoplasm of M cells, slowly entering across the M cells.

Title A comparative study of digestibility in paratuberculosis and other wasting disease in sheep

Author(s) Juste RA, Adúriz G, Bravo MV, Gonzalex L, Oregui L.

Institution SIMA, 48016 Derio (Bizkaia) SPAIN.

Abstract As loss of weight is almost the only sign of ovine PTBC, we compared the digestibility of paratuberculous sheep to that of animals affected by respiratory wasting diseases, as a means to check the importance of malabsorpiton in the pathogenesis of PTBC. The study was a conventional digestibility trial in which a total of 18 ewes were used in 3 series of 6. The digestiblity was assessed for 9-10 days, in a daily and individual basis, as the percentage of dry matter, organic matter and crude protein consumed that was not excreted by feces. The actual status of each animal was tested post-mortem on the basis of gross and microscopic lesions. Finally, only 3 animals with extensive lesions of PTBC were retained. The respiratory group was dividied in 3 animals with maedi and 3 with pulmonary adenomatosis. This, analysis of the results were carried out for 4 groups instead of 3, with only 15 ewes. The only group that gained weight throughout the experiment was the paratuberculosis one, which was the only one that consumed all the food offered. The highest loss of weight was observed in the control group, which only ate about 70% of its food. Regarding the digestibility, no significant differences between groups were found for any of the variables, being the results ranked from the best ones of the paratuberculosis group to the worst ones of the maedi group. Although a number of difficulties affected the study, our results indicate that the mere presence of extensive lesions does not interfere with digestive function of sheep. This could be in agreement with observations on the ability of PTBC diseased animals to improve their condition for short periods, and with the known lack of correlation between severity of pathological changes and clinical signs. Thus, the results of our trial, suggesting that intestinal malabsorption could not place a significant role in ovine paratuberculosis, further support the likely hypothesis of macrophage release of TNF-a being the mechanism of wasting in PTBC.

Title Prevalence of ruminant paratuberculosis in Louisiana

Author(s) Olcott BM[1], Snider TG III, Turnquist SE, Kreeger JM, Miller JE.

Institution [1]Dept. of Veterinary Clincial Sciences, School of Veterinary Medicine, Louisiana State University and A&M College, Baton Rouge, Louisiana 70803.

Abstract The prevalence of ruminant paratuberculosis has been well delineated in states of the eastern and midwestern United States. In these areas paratuberculosis is predominantly described as a disease of Holstein dairy cattle with some involvement of Bos taurus beef cattle. In Louisiana, the disease distribution is different in that paratuberculosis is seen predominantly in Bos indicus or Bos indicus crossbred beef cattle. It is seen rarely in dairy cows. The major reason for this difference in distrubiton is probably that in Louisiana, beef cattle are the predominant ruminant with approximately 6 beef cows for every dairy cow in the state. Of the beef cows, Bos indicus or Bos indicus crossbred animals are the most numerous breeds. The prevalence of paratuberculosis in Louisiana has been examined by serosurvey, by owner recognition questionnaire, and by a slaughterhouse study. Clinical case surveys and necropsy evaluations have been conducted on all animals submittted to the LSU/SVM VTH&C over the past 10 years. Prevalence, clinical and pathologic findings will be described.

Title Milk production levels in cows ELISA positive for serum antibodies to M. paratuberculosis

Author(s) Collins MT, Nordlund KV.

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Abstract Sera from 678 cows (all cows in each herd over 20 months of age) were tested for antibodies to *M. paratuberculosis* by the Johne's Absorbed EIA kit (Commonwealth Serum Laboratories, Parkville, Australia). Using the kit manufacturer's recommended cutoff we classified each cow as positive or negative. Dairy Herd Improvement records were obtained from each herd for the most recent lactation on each cow tested and transferred into a data base program. Comparison of milk production, mature equivalent 305 day lactation (ME 305), for the 43 test positive cows with their test negative herdmates revealed a 509 Kg (5.36%) lower rate of production for infected cows. The effect was minimal in lactations one and two but significant in lactation number three or greater. In addition to the reduction in milk quantity, the quality of milk from test positive cows was also less; lower protein ME 305 and lower fat ME 305. The net economic effect on productivity of cows increased with each lactation reaching over $200/test positive cow by lactation number three.

Title Epidemiological model of paratuberculosis in dairy cattle.

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Abstract A computer spreadsheet model of paratuberculosis in dairy herds was developed using Reed-Frost methods to calculate the number of new infections, and Markov chain methods to calculate culling probabilities in each time period. The model is dynamic; annual paratuberculosis prevalence rates change as infected animals join the herd or are culled. Output from the model is illustrated by graphing disease prevalence against time. Seven variables are specified at the initial stage of the model: herd size, annual herd birth rate, annual herd replacement rate, number of infected cows at time zero, number of herd replacements purchased each year, risk of purchasing a *M. paratuberculosis*-infected heifer, and number of effective cow-calf contacts per year. Sensitivity analysis of five of these variables was performed. All variables affect the course of paratuberculosis spread in herds, but the model is most sensitive to the effective contact rate. This is consistent with the findings of other infectious disease models and recommendations on paratuberculosis control: minimize cow-calf contact to prevent

transmission of the disease. The model is difficult to validate, however, because data on paratuberculosis prevalence changes in dairy herds in the absence of control measures is not available. A full description of the methods and sensitivity analysis on the model variables will appear in an upcoming issue of the Journal of Preventive Veterinary Medicine.

Title Paratuberculosis in sheep flocks: I. - Epidemiological aspects.

Author(s) Juste RA, Adúriz G, Saez de Ocariz C, Marco JC, Curevo L.

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Abstract Paratuberculosis (PTBC) represents a burden for productivity in some sheep flocks in Spain. We thought that studying the methods of diagnosis and improving our knowledge on the epidemiology of the disease in this species could help us to develop suitable measures of control. Six flocks with a history of clinical cases of PTBC were included in a follow-up program, in which several tests (immunodifusion [ID], ELISA and fecal culture) were carried out at least one per year. The size of the flocks ranged between 100 and 250 ewes, making a total of 736 animals at the beginning of the study. Up to now seven clinical cases have been reported, representing about 0.64% of the mean number of the nonvaccinated animals. Serlogical tests have been performed twice. The ID detected 3.0% the first year and 2.3% the second, for an average of 2.7%. The ELISA gave positive results in 7.9% of the animals in the first test and 28.3% in the second. Taking into account all the bands which appeared in the ID, both methods showed a significant correlation. Fecal culture, performed in 167 samples, only yielded one positive result (30 weeks of incubation). The survival rates at the second test for the ID and ELISA positive ewes, were 50.0% and 66.7% , respectively, against 66.6% and 66.1% for the negative ones. Only 50.0% of the positives in the first ID test were still positive in the second, while 77.3% of the ELISA postives in the first test remained positives in the second. Fecal culture detected less positives than ID test, and did not detect the only clinical case for which culture was performed about a year before. The ELISA, showed a sharp increase in postivity in the second year, that could not be related with any factor. Thus, with some drawbacks, the most practical test appeared to be the ID, which detected clinical cases between twelve and three months before starting symptoms.

Title Isolation of M. paratuberculosis from the environment of dairy farms with a known history of Johne's disease.

Author(s) Rosenberger AE, Whitlock RH, Siebert M, Sweeney RW, Hutchinson LJ.

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Abstract Ten dairy farms known to have had cattle with Johne's disease were evaluated for the presence of *M. paratuberculosis* in environmental samples of soil, floor scrapings, bedding and barnyard organic material. Of approximately 50 samples obtained from each farm, 15-20 were from designated locations within the barn and 20-30 from the fields, creek banks, pastures and exercise lots outside the barns. Five out of the eleven farms with a recent history of Johne's disease had positive cultures but generally with low colony counts. The highest frequency of positive cultures occurred on farms with the largest number of heavily infected cattle. The risk of environmental contamination was low on most farms with known infected cattle even if culture positive animals were present. Of the 672 total samples cultured, 3.0% yielded *M. paratuberculosis*. The most frequent positive isolation sites were from pastures and exercise lots. The maternity stall sample on the most heavily infected farm had 17-40 colonies per tube. Separation of young cattle from adult cattle potentially shedding the bacteria in the environment remains an important management recommendation.

Title Objectives and current status of the NYS Paratuberculosis Eradication and Certification Program

Author(s) Rossiter CA, Lein DH, Shin S.

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Abstract The voluntary NYS Paratuberculosis Eradication and Certification Program was started in 1985 and during 1990 had an active enrollment of 85 dairy and 15 beef farms. The program emphasizes cooperation between the herd owner, herd veterinarian, and the NYS Diagnostic Laboratory. Through biannual fecal culture testing and implementation of practical management procedures to prevent continued spread of infection, effective control programs can be designed to accommodate herds with widely varying degrees of infection. Participating herds are certified "Paratuberculosis-free" by the NYS Department of Agriculture and Markets after three consecutive negative herd tests. Owners of several severely infected herds (over 10% prevalence by fecal culture) who have actively pursued a testing and management program for 3-5 years have significantly reduced the prevalence of Johne's disease in their herds and made large improvements in overall herd productivity.

Title The Pennsylvania Johne's control program

Author(s) Whitlock RH[1], Hutchinson LJ[2], Sweeney RW[1], Van Buskirk MA[3].

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Abstract The Commonwealth of Pennsylvania has been providing a diagnostic service for farmers to help control Johne's disease for more than two decades. Annual whole herd fecal culturing, provision for indemnity payments of known infected cattle, providing advice on management methods and use of *M. paratuberculosis* vaccine in selected herds represent key elements of this program. More than 500 herds have participated in the program. The overall prevalence of Johne's disease in Pennsylvania was found to be 7.2% of culled adult dairy cattle. An estimated 20% of dairy herds in the state are believed to be infected with *M. paratuberculosis*. The annual economic losses for decreased milk production and difference in carcass weight at

slaughter exceed $5.4 million annually. Because of the great economic losses and increased demand for cattle to originate from noninfected herds, the state will implement a paratuberculosis test negative certification program in the fall of 1991. Criteria for certification will include whole herd testing of adult cattle using serum ELISA and fecal culture. Certification will be renewed annually pending negative serum ELISA and fecal culture tests.

Title Vaccination against paratuberculosis - new perspectives

Author(s) Saint-Marc B, Guillemin F, Milward F, Reynaud G, Lacoste F, Brun A.

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Abstract Vaccination is one of the necessary tools to eliminate paratuberculosis. Many countries that have eradicated tuberculosis are vaccinating against paratuberculosis with oil adjuvanted live or inactivated vaccines. Vaccination induces a good humoral and cell mediated immunity. In order to reduce the possible strong local reaction, studies were done and various vaccination schemes using different kinds of emulsions and different ways of injection were tested on sheep. It seems possible to obtain vaccination procedures providing a satisfactory immunity and an improved safety. The intradermal route has been compared to the subcutaneous route for the administration of the vaccine. Results demonstrate an improved safety and an at least equivalent activity (complement fixation test and delayed hypersensitivity) when using the intradermal route.

Title Efficiency of vaccination against paratuberculosis using an inactivated whole cell vaccine

Author(s) Wentink GH, Bongers JH, Zeeuwen AAPA, Jaartsveld FHJ.

Institution Department of Large Animal Medicine and Nutrition, P.O. Box 80.152, 3508 TD Utrecht, and Animal Health Service, Boxtel, The Netherlands.

Abstract An inactivated whole cell vaccine, containing *M. paraturberculosis* suspended in oil was administered to calves in the first month of life since 1984 in 2 farms with a culling rate for clinical paratuberculosis of 5% or more. The unvaccinated cows and calves present on the farms were the control groups. After slaughtering, the intestinal tract and adjoining lymph nodes were investigated for paratuberculosis using pathohistology, Ziehl Neelson staining and culture on Smith, and Lowestein Jensen culture tubes. The results indicate that 11.8% of the control group (340 animals) had histological and/or cultural signs of paratuberculosis, while in the vaccinated group (159 animals) only 5.0% were positive.

Title Experiences with the use of an experimental vaccine in the control of paratuberculosis in the province of Friesland, The Netherlands

Author(s) Kalis CHJ, Benedictus G.

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

Abstract As part of a national vaccination experiment, fifteen herds in the province of Friesland had their calves vaccinated since 1984. In these herds, cows born before or in 1983 served as control. These cows were not vacinated in twelve herds or vaccinated at old age in three herds. Clinical disease declined dramatically in the experimental group. From 2,069 control cows of which post-mortem examination was done, 268 (13%) had developed clinical signs of Johne's disease compared to only 13 (1%) out of 1,140 experimental vaccinated cows. Microscopic and histologic examination of the organs revealed less difference between groups with respectively 27% positive findings in control cows compared to 11% in experimental vaccinated cows. Culturing the material for the presence of *M. paratuberculosis* showed 31% positive findings in control cows compared to 38% in experimental vaccinated cows.

Title Efficiency of vaccination and other control measures estimated by fecal culturing in a regional program

Author(s) Argente G.

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Abstract Bovine paratuberculosis FGDS22 - Control program in Cotes de'Amor, France uses fecal culture in high clinical incidence herds to cull infected cattle before clinical onset. Many thousands of cattle have been cultured one or more times since 1982. The first two tests and cull operations in infected herds at 1 year intervals have the best cost/benefit ratio to lower clinical rate and occurrence of persistent excretors which are the source of infections for calves. The cultures performed in the following years are less useful. Statistical results are presented according to the age of cattle, for cattle born before or after the beginning of hygienic control measures in the herds and for cattle vaccinated or not. Vaccination appears to be the key factor of the control program.

Title Skin test for Johne's disease after vaccination against paratuberculosis: relationship with postmortem findings.

Author(s) Wentink GH, Bongers JH, Zeeuwen AAPA.

Institution Department of Large Animal Medicine and Nutrition, P.O. Box 80.152, 3508 TD Utrecht, and Animal Health Service, Boxtel, The Netherlands.

Abstract After vaccination against paratuberculosis, using a whole cell inactivated vaccine containing *M. paratuberculosis* suspended in oil in the first month of life, an intradermal skin test with Johnin was done between the 6th and 12th month of life. After postmortem examinations on 154 animals, the results indicate that the stronger skin reactions occurred only in animals that were free of paratuberculosis using pathohistological, acid fast staining and cultural techniques, and that negative results in the skin test occurred relatively more frequently in animals found positive in postmortem examinations.

Title Paratuberculosis in sheep flocks: II. - Vaccination.

Author(s) Adúriz G, Juste RA, Saez de Ocariz C.

Institution SIMA, 48016 Derio (Bizkaia) SPAIN.

Abstract The extensive use of vaccination should be supported by some knowledge on the protection afforded, the possibilities of use in adult sheep and its effects on immunological tests. In this paper we will present preliminary results on some of those aspects, obtained in a field study with an attenuated vaccine widely using in Spain. The study has been carried out for more than two years on six sheep flocks in which annual incidence of clinical cases ranged between 0.5% and 6%. The vaccine was applied to about a half of the replacer ewe lambs of each flock at about one month of age. Moreover, half of the adult ewes were also vaccinated with the same dose. Before vaccination and about six months afterwards, immunodiffusion (ID) and ELISA were carried out on the replacers. Once per year all ewes older than a year were submitted to ELISA and ID. Up to now only two non-vaccinated ewes from the control group have appeared with clinical signs of PTBC. There was a significant correlation between the results of ID and ELISA of the lamb before vaccination and those of its dam. There was also a correlation between results at birth and at six months, both in vaccinated and in non-vaccinated lambs. The first group of vaccinated replacer ewes showed a decrease in the frequency of positives in the ID test from 48.2%, about 13 months after vaccination, to 13.2% at 23 months post-vaccination. The group vaccinated in the following year had 37.2% reactors by the 6th month and 26.9% by the 9th month. The control groups changed from 0.0% to 1.4% and 3.2%, respectively. The first group of ewes vaccinated as adults were 15.0% positive by the 18th month post-vaccination, while the second one were 9.9% positive by the 6th. In the ELISA, all the groups appeared to keep high levels of reactivity (about 90%) at all the times. It could be pointed out that transfer of

humoral immunity by colostrum seems to occur, which apparently does not interfere with response to vaccination. The relatively fast decline in the percentage of positives in the ID test after vaccination, contrasts with the maintenance of high levels in the ELISA.

Title Efficacy of two associations of antituberculous drugs in calves experimentally infected with Mycobacterium paratuberculosis.

Author(s) Belloli A, Arrigoni N, Vacirca G, Agosti A, Pozza O, Greppi G, Proverbio D.

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Abstract Two associations of antituberculous drugs were tested for their ability to prevent infection in calves orally inoculated with *Mycobacterium paratuberculosis* in the first few days of life. The associations were: rifampin (RMP) + clofazimine (CFA) and rifampicin (RMP) + streptomycin (SM) + pyrazinamide (PZA). Drugs were administered daily in the milk, for a period of seven months, starting the day after experimental inoculation. The RMP+SM+PZA association prevented the intestinal colonization and infection was avoided, while RMP+CFA was only able to delay *M. paratuberculosis* excretion in the feces, in comparison to controls.