The Paratuberculosis Newsletter

The official publication of the International Association for Paratuberculosis

INSIDE THIS ISSUE

Note from the Editor

New IAP website

Upcoming Events

Contributions by IAP Members

Recent Paratuberculosis Literature



Note from the Editor

Dear IAP members,

Here we go with the third issue of the Paratuberculosis Newsletter in 2025!

In previous issues we have informed on the plan that the Communication Committee has traced to enhance communication reinforcing old channels (newsletters and association's website) and creating new ones (social media). In this issue, Peter Orpin will further comment on the new website (see pages 3-4).

In the Upcoming Events section, updates on our next ICP in Germany and dates to be saved from other conferences that can be interesting for paraTB researchers (see page 5).

In this issue, again, we are very pleased to have contributions from IAP members. First, Dr. Ramón Juste, past President (2007-2014) and current Board member with a long-standing science record on paratuberculosis research that contributes to this issue with a comment on control by fecal PCR (see page 6) and a description of a paraTB control plan designed in Spain (see pages 7-12). In the Technology updates section a short piece on a novel digital PCR for Map detection from IAP member Eric Sellal (see pages 13-14). Finally, a short summary of work on paraTB that has been presented at the International Veterinary Immunology Symposium 2025 in Vienna this August by Dr. Antonio Facciuolo and myself (see pages 15-16). Please don't miss all these submissions!

Catching up with paratuberculosis research literature is getting tough! Over 30 new papers have been published since June 2025. (see pages 18-20).

As always, the Association's Officers and Board members are happy to receive suggestions and feedback from you to help strengthen our community.

Best wishes.

Natalia Elguezabal

Contribute with your photos



Please consider sharing photographs you have for publication in the next Newsletter! Let's display the diversity in ruminants, their environments, and where our members are conducting research, science outreach or just having a nice walk.

Please help me! I'm running out of pictures while you are learning a lot about Basque ruminant breeds.

Cover photograph: Azpi gorri breed goats at a Livestock Fair, Mungia, Bizkaia (Basque Country). *Azpi gorri* comes from Basque meaning beneath 2 or under= azpi and red= gorri.

New IAP website

By Peter Orpin

This autumn we will launch a new website for the Association.

The platform has been refreshed and is based on a more familiar WordPress software. The benefit of this is that it will be easier for the newly formed Communication group, led by Alejandra Colombatti, to keep the site up to date.

The key aim of the website is to promote the activities of the Association by providing a central point of information for members and prospective members.

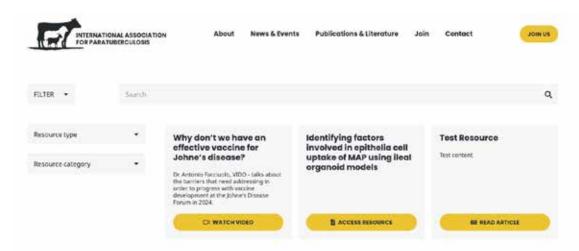
So, what is new and improved?

 The website look and feel has been modernised completely with a more inclusive logo.



- The website can now be immediately translated into 10 different languages.
- An expanded database behind the website to allow you to both register your interests (for more selective email contact) and, also for us to filter content according to interests
- The re-introduction of a members' forum with pre-set groups which can allow members to ask questions and seek answers.
- The further development of a **Member Resources** section which allows us to host and categorise videos, pdfs and articles. The aim of this is to provide an enhanced information flow through the website.

New IAP website (continued)



 The newsletters are now archived and accessible. Considerable time is taken generating this resource and this allows us to showcase our activity to both members and non-members. Only members will be allowed to access the most recent newsletters.



• Improved payment options for members utilising Paypal and Card payments

This is very much a work in progress but hopefully this will help bridge the communication gap highlighted in the member survey we conducted in 2024.

If you have any content that you believe would be helpful to include in the Member Resources (Videos of talks/ presentations, articles or information on your own control programmes) then please get in touch with myself (pete.orpin@outlook.com) or Alejandra (macolombatti@gmail.com). We will send further prompts to encourage you to engage with the website once it is launched.

Thanks again for your feedback that helped shape the website and look forward to seeing how it can be utilised to help you with your work on paratuberculosis.

Upcoming Events

In the next months many attractive meetings where we can share our advances in paratuberculosis, learn from others, and raise awareness on this disease will be celebrated. Check out this selection.

4th International Precision Dairy Farming Conference. 3-5 December 2025. New Zealand. (https://www.precisiondairyfarmingconference.nz/)

EAAP-ASAS Conference on Livestock farming and the environment: emissions and solutions. 19-21 April, 2026. Azores Islands, Portugal. (https://asas.eaap.org/).

ADSA 2026 Annual meeting. 21-24 June, 2026. Milwaukee, Wisconsin. (https://www.adsa.org/Meetings/2026-Annual-Meeting).



33rd WBC. WORLD BUIATRICS CONFERENCE. 6-10 September, 2026. Istanbul, Turkey. (https://www.wbc2026istanbul.com/).

9th European Veterinary Immunology Workshop. 8-10 September, 2027.
Florence, Italy. SAVE THE DATE

5



ICP 2026 Germany

ICP 2026 will be hosted in the city of Dresden, Germany. The meeting will take place from June 7th to 11th, 2026 in the German Hygiene Museum. The colloquim is being organized by Heike Kohler, Karsten Donat and Susanne Eisenberg.

The official conference website (icp2026.com), is now live and features updated information regarding keynote speakers, the scientific advisory board, and abstract submission guidelines.

We strongly encourage all prospective participants to subscribe to the **ICP 2026 newsletter** via the website to be informed about this event.

Please note that the deadline for abstract submission is **December 15, 2025**.

We look forward to seeing you in Dresden in 2026 for what promises to be an enriching and memorable scientific gathering.

Test and Cull (and vaccination) with Fecal PCR Comment

By Ramón Juste

I was delighted to read Richard's comment on their paper on paratuberculosis control using fecal PCR, as it multiplied my excitement back in Vrindavan at the IDF forum and ICP when Anne Klassen made her presentations. It might not be necessary to rush into buying fecal PCR kits, but I think it is urgent to review the use of ELISA and, in general, the approach to paratuberculosis control in the light of recent knowledge.

As we have informed at the successive Colloquia and IDF fora, here in the Basque Country (Spain), we have been running a control program comparing test and cull (T&C) with vaccination. Although we started limited testing over 20 years ago, we could not involve the local and national animal health authorities until 2011 to extensively work with affected farms. Since then, we have been comparing our strategies' results for 15 years in a total of 30 cattle herds, accounting to over 8000 cattle and nearly 30000 individual records. Keeping the 28 herds that remained in the program for more than three consecutive years and following a whole vaccinated versus control herd design to account for the population effect of vaccination, we have followed 7 farms on a test and cull strategy and 21 on a vaccination program. As we are currently writing the report to formally publish the results, I cannot help but to concur with Richard's enthusiasm on the efficacy of the fecal PCR that, in agreement with results in Germany, reveal the possibility of eradicating paratuberculosis in less than 10 years.

In our case, in addition to extending the follow up to 14 years, we have observed similar results in a larger number of individual herds. Briefly, while fecal PCR cleared shedding in all herds by the 12th years in the T&C group and by the 9th year in the vaccinated herds, ELISA-based testing kept positive in herds until the last 14th year. Actually, we have gone one step further since we demonstrate that fecal PCR testing is fully compatible with vaccination and that by combining both, it should be possible to reduce time to last positive control by 3 years, and reducing testing costs by prolonging the lag between tests to two or even three years since all animals would be protected by increased resistance and reduced environmental contamination.

All these results call to quickly switch from ELISA to extensive fecal PCR testing, thus recovering the old fecal detection strategy proposed in the early paratuberculosis times, since, according to the literature, during similar extents of time, the former can reach only a moderate prevalence reduction, while the latter seems to consistent and persistently stop shedding especially when combined with vaccination, the other old neglected tool.

Based on these premises, we proposed to the Spanish Ministry of Agriculture the Paratuberculosis program that we summarize in this issue and that represents the closing of a research cycle in my scientific career in SIMA/NEIKER that was triggered by the Basque animal health administration question on how to deal with the first case of bovine paratuberculosis in Spain 42 years ago.

Paratuberculosis control plan in Spain

By Ramón A. Juste and Joseba M. Garrido

NEIKER-BRTA Instituto Vasco de Investigación y Desarrollo Agrario, Animal Health Department, 48160 Derio, Bizkaia, Spain

Introduction

In Spain, paratuberculosis (PTB) was first mentioned in a clinical case report in cattle in 1935 on the grounds of a diarrhea with possible acid-fast bacilli in feces, but complete healing with an iron treatment (Roca Soler, 1935). The first well documented cases occurred in dairy sheep in Leon in 1972 (Aller Gancedo et al., 1973) and in Charolais cattle in Bizkaia (Juste et al., 1983). Currently, it can be considered widely distributed as the latest large slaughterhouse study showed (Vazguez et al., 2014). However, as in the rest of the world, it is difficult to assess its prevalence and real impact on production. Although paratuberculosis is one of the diseases that raises most concern among livestock farmers, there was no specific and general control program for PTB in Spain. Instead, there are different voluntary control plans based on test-andcull (T&C) strategies and good management practices carried out by cattle, sheep and goats' health defense groups, which are subsidized by regional administrations (Whittington et al., 2019). Although cost-benefit analyses (Cho et al., 2012; Juste and Casal, 1993), show that vaccination against PTB is the best way of controlling the disease and, in Spain, it is widely practiced in dairy sheep and goats, its use in cattle has been restricted to a field trial in the Basque Country in farms with a history of heavy clinical incidence that has lasted 15 years. In it, it was shown that paratuberculosis was associated with an 8% milk production drop and that MAP shedding could be stopped in less than 10 years of annual replacers vaccination or T&C (Figure 1).

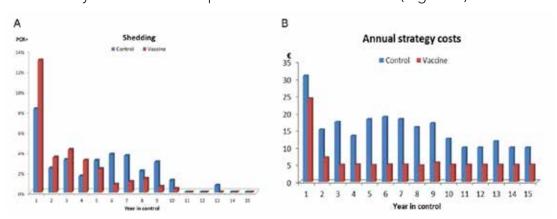


Figure 1. Evolution of shedding and costs in the Basque Country paratuberculosis control trial. A. Percentage of MAP shedders per group detected by real-time PCR in each of the annual samplings. After Year 8 in TCS herds and Year 11 in VS herds less than 500 animals were tested annually. B. Estimated annual costs of each strategy.

This time-to-shedding eradication was matched with the T&C reference strategy using fecal PCR confirmation, but with at least one case of shedding in the following 5 years and at a much lower benefit/cost ratio (Alonso-Hearn et al., 2012; Garrido and Juste, 2023; Juste et al., 2009) (Figure 2).

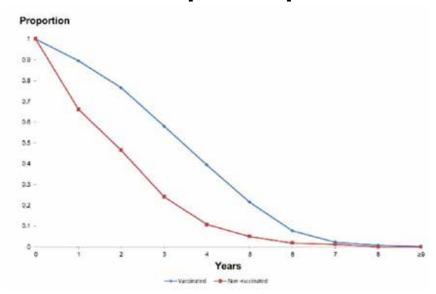


Figure 2. Survival curves for vaccinated and control calves grouped in one-year classes. Maximum differences were observed between 1 and 5 years of age. They start below and then overlap the paratuberculosis clinical presentation lower age range. Sidak logrank test p value < 0.0001.

It should be noted that while the direct MAP infection indicator fecal PCR showed a consistent evolution compatible with vaccination and leading to full shedding clearance, the indirect ELISA testing did not match these results and, in addition to be unusable on vaccinated herds, continued yielding positive results until the last control in T&C herds (unpublished results). Recent publications confirm this perspective on the efficacy of fecal PCR for paratuberculosis control in cattle (Donat et al., 2024; Garcia et al., 2025).

The recent proposal of the "trained immunity" non-specific effects of BCG vaccination in children provided the grounds to examine the hypothesis of a similar effect in the field trial that showed that survival could be increased by up to 26% with paratuberculosis vaccination (Juste et al., 2021) (Figure 3). Adding these benefits to the fact that paratuberculosis vaccination can be considered as heterologous regarding bovine tuberculosis and can be easily differentiated from true TB infection, we think that vaccination should be taken into account and included in paratuberculosis control programs.

Based in the scientific literature and our own experience we have written a paratuberculosis control program draft that has been submitted to the Spanish Ministry of agriculture for use as the common grounds for paratuberculosis control in the different susceptible species in each Autonomous Community (AC) in which the Spanish territory is administratively divided.

The bibliography includes works related to the development of an algorithm that allows to determine the risk of developing the advanced forms of paratuberculosis that are responsible for the clinical loses (Badia-Bringué et al., 2023, 2024; Canive, Badia-Bringué, et al., 2021; Canive et al., 2022) that has raised the interest of the Spanish dairy cattle association and whose underlying polymorphisms have already been included in the standard genotyping medium density chip.

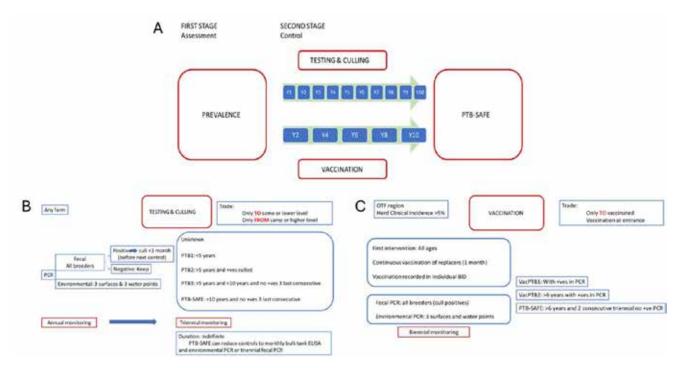


Figure 3. Paratuberculosis control program. A. Overall dynamics. B. Testing and culling procedure and scoring. C. Vaccination procedure and scoring.

CONTROL PROGRAM

The control program is founded on a holistic perspective and is focused on progressive reduction of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in the different susceptible species since it is assumed that MAP cannot be eradicated from the environment. The Spanish Ministry of Agriculture will coordinate the AC animal health authorities which according to Spanish de-centralized administration, are the ones in charge of animal health management. Therefore, the control program will define paratuberculosis cases at the individual level according to the WOAH definition and at the regional level according to prevalence surveys and propose reduction strategies for each species to be implemented by each one of the AC.

Paratuberculosis, according to the WOAH and for the purposes of notification to the WOAH, is defined as an infection of domestic and captive wild ruminants from the families Bovidae, Camelidae and Cervidae with MAP. More specifically, a case of paratuberculosis occurs when there is: a) an isolation of MAP; b) a nucleic acid sequence specific to MAP detection or c) a non-vaccinal MAP-specific antibodies demonstration in samples from an animal with clinical signs and pathological lesions that has been epidemiologically linked to a confirmed case of infection with MAP o there is a cause to suspect that the animal has previously been associated or had contact with materials containing MAP.

For paratuberculosis control purposes, a farm that has at least one animal matching this definition will be an outbreak of paratuberculosis. The Spanish control program proposes two stages. The first one is prevalence assessment and the second is control program implementation.

Prevalence assessment

For domestic species prevalence assessment should require the investigation of, at least, 30 heads in each one of 100 farms representative of production system and territory of the corresponding AC.

For wildlife, the prevalence estimate would be made by fecal PCR testing for paratuberculosis in the samples collected within the wildlife tuberculosis and brucellosis surveillance (PATUBES) program. If (preferentially ileal and jejunal) lymph nodes are available, confirmation could be obtained by isolation or PCR.

This information will only have statistical and scientific value and individual farm results will not be public.

Control program

The control program will be voluntary and will be based on keeping an official record of participating farms strategy choice, culling and replacement and PTB prevalence.

Bovines (Bos taurus, Bos indicus, Bubalus bubalus)

Two strategies can be used: T&C and vaccination. Both can reduce clinical cases from the beginning of the control, but the costs are higher for the T&C approach. Each farm must choose a strategy (figure 3).

In officially tuberculosis free (OTF) regions both strategies can be applied. For T&C, all animals aged 12 months or more should be submitted to a fecal PCR with killing of positives in less than one month. According to time and results, five categories will be recognized. Unknown: No confirmatory tests have been carried out. PTB1: The herd has been annually tested between 1 and 4 years and all positive animals have been culled. PTB2: The program has been carried out for 5 and 8 years and all positive animals have been culled. PTB3: The herd has been submitted to annual testing for more than 4 years and no positive results have been found in the last two consecutive years. PTB4: The herd has been submitted to annual testing for more than 5 years and no positive animals have been found in the last three consecutive controls. Farms in status PTB4 can reduce controls to semiannual antibody detection in bulk tank milk or in an annual blood sample of not less than 10 animals in order to keep that status. Herds in the higher status levels (PTB3 and PTB4) cannot introduce animals from farms with lower levels. Farms with lower status should only buy animals from farms with higher status but can also exchange animals within their own level. These farms can never introduce vaccinated animals.

Only farms with an initial prevalence higher than 5% by any method and where the local animal health authority have assessed its compatibility with the national bovine TB erradication program are eligible for vaccination. At the start of the program, all animals older than one month than are going to be kept in the farm should be vaccinated. Vaccination records regarding brand, lot date and age at vaccination should be kept by the farmer. After vaccination, confirmation by fecal PCR is necessary upon which positive animals must be culled as soon as possible. Vaccination of replacers should be indefinitely maintained.

Vaccinated farms will be classified as follows. VacPTB1 farm: Farms that have been vaccinating for less than 3 years. VacPTB2 vaccinating-negative farms: Farms that have been vaccinating for more than 3 years and are negative in fecal and environmental PCR. VacPTB safe farms: Farms that have been vaccinating for, at least 6 years and are negative in the two last testings and keep vaccinating. VacPTB negative farms: Farms that have stopped vaccination and remain negative in periodic fecal and environmental MAP-PCR. Vaccinating farms can exchange animals only with other farms in vaccination but cannot sell for life to herds in T&C. The farm can acquire animals from T&C at any stage that will be vaccinated at entrance.

In regions not officially tuberculosis free only T&C would be allowed except in special cases such as herds of special genetic value with a risk of extinction or in any other situations that the local competent authority deems necessary to grant a special permit.

Farms within this strategy can only be submitted to comparative cervical skin test (Serrano, et al., 2017) in the national TB bovine erradication program and must be very wary of possible cases. The IFN γ release assay (IGRA) should not be used in vaccinated animals.

Once developed, breeding criteria for PTB resistance (Badia-Bringué et al., 2023, 2024; Canive, Badia-Bringué, et al., 2021; Canive, González-Recio, et al., 2021) it will be possible to add this feature to breeding programs by including the markers in the genotyping protocol and using an algorithm to favor selection of resistant genotypes and rejection of the more susceptible to clinical forms.

Small ruminants

Given the higher relative individual costs of testing and while it does not interfere with tuberculosis control immune tests, only vaccination can be considered an efficient strategy. In fact, this strategy is already extensively in use in several regions.

Other species (camelines, cervines)

Taking into account the limited availability of reagents for diagnostics in these species, only vaccination is considered for them. It must be carried out with the same criteria and specifications as for small ruminants unless they belong to zoologic establishments in TB non-negative control programs. In any case, they would be subject to general legislation on tuberculosis control (RD 138/2020 y Reglamento 688/2020).

Wildlife

The leporine species are considered as end hosts for paratuberculosis. As a rule, it would be necessary to act only on the domestic species to which they are linked in case of PTB detection in these species. Nevertheless, in cases where there are serious suspicions of having become a risk for the domestic species, separation, population regulation or other measures, potentially including immunization, must be assessed.

Other wild species (suines, cervines, caprines, ovines) do not seem to play a relevant role in the epidemiology of paratuberculosis of domestic species. In case they showed indications of doing it, vaccination would be the choice for control.

Exotic species

In general, they are restricted to small collections but high individual value that are kept in isolation and do not pose any risk for domestic species. In case of necessity, they could be dealt with vaccination and culling of advance clinical cases.

In case of declaration of tuberculosis outbreaks in the farms, zoologic centers or any populations in a paratuberculosis control program, priority will be given to control of tuberculosis.

References

- -Aller Gancedo, B., Fernandez-Diez, M., and Escudero-Diez, A. (1973). "Paratuberculosis Ovina". Suplemento Cientifico Del Boletin Informativo Del Consejo General de Colegios Veterinarios de España, 196, 11–18.
- -Alonso-Hearn, M., Molina, E., Geijo, M., Vazquez, P., Sevilla, I. A., Garrido, J. M., and Juste, R. A. (2012). "Immunization Of Adult Dairy Cattle With A New Heat-Killed Vaccine Is Associated With Longer Productive Life Prior To Cows Being Sent To Slaughter With Suspected Paratuberculosis". *Journal of Dairy Science*, 95(2), 619–629. https://doi.org/10.3168/jds.2009-2860
- -Badia-Bringué, G., Canive, M., and Alonso-Hearn, M. (2023). "Control Of *Mycobacterium Avium* Subsp. *Paratuberculosis* Load Within Infected Bovine Monocyte-Derived Macrophages Is Associated With Host Genetics". *Frontiers in Immunology*, *14*. https://doi.org/10.3389/FIMMU.2023.1042638
- -Badia-Bringué, G., Canive, M., Vázquez, P., Garrido, J. M., Fernández, A., Juste, R. A., Jiménez, J. A., González-Recio, O., and Alonso-Hearn, M. (2024). "Genome-Wide Association Study Reveals Quantitative Trait Loci And Candidate Genes Associated With High Interferon-Gamma Production In Holstein Cattle Naturally Infected With *Mycobacterium Bovis*". International Journal of Molecular Sciences 2024, Vol. 25, Page 6165, 25(11), 6165. https://doi.org/10.3390/IJMS25116165
- -Canive, M., Badia-Bringué, G., Vázquez, P., Garrido, J. M., Juste, R. A., Fernandez, A., González-Recio, O., and Alonso-Hearn, M. (2022). "A Genome-Wide Association Study For Tolerance To Paratuberculosis Identifies Candidate Genes Involved In DNA Packaging, DNA Damage Repair, Innate Immunity, And Pathogen Persistence". Frontiers in Immunology, 13, 1. https://doi.org/10.3389/FIMMU.2022.820965/PDF
- -Canive, M., Badia-Bringué, G., Vázquez, P., González-Recio, O., Fernández, A., Garrido, J. M., Juste, R. A., and Alonso-Hearn, M. (2021). "Identification Of Loci Associated With Pathological Outcomes In Holstein Cattle Infected With *Mycobacterium Avium* Subsp. *Paratuberculosis* Using Whole-Genome Sequence Data". Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-99672-4
- -Canive, M., González-Recio, O., Fernández, A., Vázquez, P., Badia-Bringué, G., Lavín, J. L., Garrido, J. M., Juste, R. A., and Alonso-Hearn, M. (2021). "Identification Of Loci Associated With Susceptibility To *Mycobacterium Avium* Subsp. *Paratuberculosis* Infection In Holstein Cattle Using Combinations Of Diagnostic Tests And Imputed Whole-Genome Sequence Data". PLoS ONE, 16(8 August). https://doi.org/10.1371/journal.pone.0256091
- -Cho, J., Tauer, L. W., Schukken, Y. H., Gomez, M. I., Smith, R. L., Lu, Z., and Grohn, Y. T. (2012). "Economic Analysis Of *Mycobacterium Avium* Subspecies *Paratuberculosis* Vaccines In Dairy Herds". J.Dairy Sci., 95(1525-3198 (Electronic)), 1855–1872.
- -Donat, K., Einax, E., Rath, D., and Klassen, A. (2024). "Successful Control Of *Mycobacterium Avium* Subspecies *Paratuberculosis* Infection In A Dairy Herd Within A Decade-A Case Study". Animals: An Open Access Journal from MDPI, 14(6). https://doi.org/10.3390/ANI14060984
- Garcia, A. A., Plain, K. M., Toribio, J.-A. L. M. L., and Whittington, R. J. (2025). "Evaluation Of A Test-And-Cull Strategy To Control Bovine Paratuberculosis Based On The Detection Of *Mycobacterium Avium* Ssp. *Paratuberculosis* DNA Using The HT-J Fecal PCR Test". Journal of Dairy Science. https://doi.org/10.3168/JDS.2024-25877
- -Garrido, J. and Juste, R. (2023, March 20). "8. The Whole Story Of The Long-Term Vaccination Trial In Dairy Cattle In The Basque Country. What About Non-Specific Effects?". Proceedings of the 7th and 8th IDF Paratuberculosis Fora. https://doi.org/10.56169/UEAO1249
- -Juste, R. A., Alonso-Hearn, M., Molina, E., Geijo, M., Vazquez, P., Sevilla, I. A., and Garrido, J. M. (2009). "Significant Reduction In Bacterial Shedding And Improvement In Milk Production In Dairy Farms After The Use Of A New Inactivated Paratuberculosis Vaccine In A Field Trial". BMC Research Notes, 2. https://doi.org/10.1186/1756-0500-2-233

Technology updates

Innovations in the Diagnosis of Paratuberculosis: Development of New Tools for Accurate Detection and Quantification of *Mycobacterium avium* subsp. paratuberculosis (MAP)

Amandine Berthet (amandine.berthet@biosellal.com), Aurélie Plantecôte (aurélie.plantecote@biosellal.com), Jerôme Ventosa (jerome.ventosa@biosellal.com) and Eric Sellal (eric.sellal@biosellal.com)

Introduction

Paratuberculosis, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a chronicenteric disease in ruminants leading to significant economic losses. A threshold of 104 MAP/gram of feces is considered critical for distinguishing active infections from passive shedding. This data is crucial for controlling and managing this disease through regular herd surveillance and accurate diagnostic methods like quantitative PCR (qPCR). Most of the existing qPCR assays, targeting the IS900 gene, can result in inaccurate quantifications due to a high variability in gene copy numbers across bacteria.

Methods

In collaboration with Labocéa (Quimper, France), BioSellal has developed and validated new digital PCR (dPCR) and qPCR workflows using a Reference Material (RM) calibrated based on the single-copy F57 gene. These methods are designed for use with both fecal and environmental samples and comply with French standards (NF U47-600) and MIQE guidelines. The dPCR solution provides accurate MAP quantifications, while the qPCR method offers relative quantification based on the RM.

These tools are currently being applied in field studies across several French departments. An initial study in the Vosges region compared qPCR and dPCR on different types of samples from cattle, sheep and goat herds. A more extensive study is scheduled for autumn 2025 in Brittany, where all animals from four cattle farms, along with environmental samples, will be analyzed using all developed products.

Results & Discussion

We have developed the RM and two quantification methods: a direct dPCR method quantifying IS900 and F57, and a qPCR RM-based relative quantification method with the same targets. Results show significant advantages in combining IS900 for sensitivity and F57 for specificity and accurate quantification. The RM, calibrated at 104 MAP bacteria/gram, proved to be reliable for harmonizing and comparing results through different extraction and PCR methods. The dPCR method is proving particularly reliable in livestock environments, thanks to its enhanced inhibitors resistance and its accuracy, both of which help to better establish herd status.

Technology updates (continued)

Analyses carried out during a field study in the Vosges region have enabled us to assess the relevance of analyzing faecal mixtures in the environment of livestock herds using different methods. The study compared qPCR and dPCR quantification or detection for 300 samples from around 120 livestock farms. It showed that dPCR is more tolerant to inhibitors for this type of sample, which is particularly rich in inhibitors. In addition, dPCR allows absolute quantification of the bacterial load while combining specificity and sensitivity thanks to the detection of both MAP targets IS900 and F57. This precise and reproducible absolute quantification is a major asset, particularly for longitudinal monitoring of farm status.

All data from ongoing field studies will be shared through scientific communications and publications.

Conclusion

These innovative qPCR and dPCR tools offer enhanced accuracy in paratuberculosis diagnostics. Their integration into routine herd surveillance could improve disease management and reduce the economic impact on livestock industries.



Paratuberculosis at IVIS 2025

By Natalia Elguezabal and Antonio Facciuolo

The 14th International Veterinary Immunology Symposium (IVIS), took place in Vienna from 11-14 August 2025. Over 300 delegates from more than 50 different countries attended. IVIS is a global scientific meeting held every three years for veterinary immunologists and researchers to share cutting-edge research on animal health, focusing on the immune system's role in health and disease. The symposium features presentations, workshops, and networking opportunities to discuss topics like vaccine developmente, host-pathogen interactions, zoonotic diseases, and disease control strategies in various animal species. It serves as a vital platform for collaboration and the exchange of ideas to advance the field of veterinary immunology and address global health challenges through a One-Health perspective.

In this edition, many infectious diseases that affect animals were covered and of course there was space for our beloved paraTB. Here a brief summary on what was presented.

In the **Innate Immunity** section, Maitane Mugica from NEIKER (Basque Country, Spain) delivered an oral presentation titled "The role of neutrophils in vaccination against paratuberculosis: evidence of activation and trained immunity" where she talked about the positive effects of immunization with an inactivated Map vaccine on the activity of neutrophils in calves and how this immunization displays features of trained immunity and potentially exerts heterologous protection against other pathogens that impact livestock such as Escherichia coli and Staphylococcus aureus. Also, in the innate immunity session, Dr. Noive Arteche-Villasol from the University of León (Spain) shared her work on the immunomodulation that paraTB vaccination triggers on caprine monocyte-derived macrophages and how vaccination increases the phagocytic and bactericidal activity of these phagocytes ex vivo in a poster titled "Modulation of monocyte-derived macrophage immune response following paratuberculosis vaccination in goats". Finally, Dr. Natalia Elguezabal (Spain) presented the poster "The role of neutrophils in paratuberculosis: a focus on efferocytosis". This study describes efferocytosis of bovine neutrophils by bovine monocyte derived macrophages ex vivo using in vivo imaging technology finding different efferocytosis rates depending on neutrophil stimulation with Map or BCG indicating possible differences on how neutrophils themselves or in cooperation with macrophages behave upon encounter with these mycobacteria.



In the Tissue Specific Immunology / Microbiome session Dr. Antonio Facciuolo from VIDO (Canada) delivered an oral presentation entitled "Application of a bovine intestinal loop model to study in vivo mucosal immune responses to enteric Mycobacterium Infection". ln this talk, Tony described two functionally distinct types of Peyer's patches (PP) in ruminants; discrete PPs dispersed throughout the

Paratuberculosis at IVIS 2025 (continued)

jejunum and the single continuous PP that occupies the ileum. Using a surgical model his group prepares intestinal segments ('gut loops') in young calves to target MAP to these individual PPs to interrogate tissue-specific immunity. His groups has found marked differences in MAP persistence, infection-induced immunity, and parenteral vaccine-induced immunity between these intestinal sites. Using this approach they have started to define mucosal correlates of protection for enteric MAP infection and revealed how parenteral vaccination impacts intestinal immunity in the ruminant small intestine.

Also, in this session, Maitane Mugica from NEIKER presented a study performed at the Royal Veterinary College (London) on an alternative model to study host-pathogen interaction in the intestine with a poster entitled "Bovine precision cut intestinal slices as a model to assess early events between intestinal tissue and *Mycobacterium avium* subsp. *paratuberculosis*". Here, a description of the optimization of the culture of precision cut intestinal slices and some preliminary results on Map infection of these slices were disseminated.

Next IVIS will be celebrated in Montreal in 2028. Let's hope for immunological innovations in paraTB in the next few years that can be presented there!

Meanwhile if you have a special interest or questions on any of these contributions you can contact us and we will be happy to expand on them. Natalia Elguezabal: nelguezabal@neiker.eus, Antonio Facciuolo: antonio.facciuolo@usask.ca





Maitane Mugica (left) and Dr. Antonio Facciuolo (right) delivering their oral presentations at IVSI2025 in Vienna.

Officers of the Association



President: Antonio Facciuolo

Vice-President: Natalia Elguezabal

Secretary-Treasurer: Adel Talaat

Board Members: Alejandra Colombattii, Sam Strain, Peter Orpin, Ramón Juste

Past-President: Conor McAloon

Contribute to the Next Newsletter

The next newsletter will be published in December 2025.

We invite all members to contribute by sharing opinion pieces, news about recent publications or project updates, regional control programs, job opportunities, or any relevant information on paratuberculosis research and communications presented at meetings.



17

Please submit your article to:

newsletter@paratuberculosis.com; nelguezabal@neiker.eus

Submission deadline for the upcoming issue is November 30th, 2025.

We welcome all submissions!

Some of the images included in this issue have been generated with Leonardo AI (LAI).



Recent Literature on MAP

Research papers focused on *Mycobacterium* avium subsp. paratuberculosis published during the past three months have been included in the following list.

Links to the open access versions of the papers have been included in the titles when possible.

Enjoy reading!

Colombatti Olivieri MA, Moyano RD, Nagel A, Gravisaco MJ, Delgado FO, Alvarado Pinedo MF, Travería GE, Alonso MN. <u>Attenuation of a local Mycobacterium avium subsp paratuberculosis virulent strain by homologous recombination</u>. Res Vet Sci. 2025 Jun 13;193:105758. doi: 10.1016/j.rvsc.2025.105758.

Ozturk M, Dortbudak MB, Bekmez B, Biagini L, Altuğ N, Rossi G, Ozturk Y, Di Cerbo A. Oxidative Stress and Apoptotic Markers in Goats Naturally Infected with Mycobacterium avium subsp. paratuberculosis. Pathogens. 2025 Jun 16;14(6):593. doi: 10.3390/pathogens14060593.

Holder T, Robinson N, Jones GJ. <u>The Impact of Bacillus Calmette-Guérin Vaccination and Mycobacterium bovis Infection on Diagnostic Antibody Tests for Mycobacterial Infections.</u> Vaccines (Basel). 2025 May 28;13(6):578. doi: 10.3390/vaccines13060578.

Arrazuria R, Oyanguren M, Molina E, Mugica M, Gunapati B, Subbian S, Lavin JL, Anguita J, Elguezabal N. Dual effects of probiotic administration prior to *Mycobacterium avium* subsp. *paratuberculosis* infection are associated with immunological and microbiota shifts. Sci Rep. 2025 Jul 2;15(1):23341. doi: 10.1038/s41598-025-06860-7.

Murato Y, Hayama Y, Kondo S, Yamaguchi E, Yamamoto T. <u>Estimation of the risk of cohabitation with Johne's disease-infected cattle in Japanese dairy cows.</u> Prev Vet Med. 2025 Oct;243:106611. doi: 10.1016/j.prevetmed.2025.106611.

Vass-Bognár B, Bakony M, Fornyos K, Baumgartner W, Khol JL, Jurkovich V. When is the best time to test paratuberculosis positivity? Observations from a follow-up study in Hungarian dairy herds. Front Vet Sci. 2025 Jun 19;12:1570915. doi: 10.3389/fvets.2025.1570915.

Contreras-Vilchez VE, Mendoza-Palomino E, Jiménez-Aparco C, Huamán-Lizana D, Acuña-Leiva A, Quispe-Ccasa HA. Cows of reproductive age in the high Andean region of Peru have seroprevalence of antibodies against bovine herpesvirus 1 and *Neospora caninum*. Am J Vet Res. 2025 Jul 8:1-9. doi: 10.2460/ajvr.25.04.0141.

Badia-Bringué G, Alonso-Hearn M. RNA-sequencing studies suggest that microRNAs and alternative splicing of pre-mRNAs modulate immune and inflammatory responses in Holstein cattle infected with *Mycobacterium avium* subsp. *paratuberculosis*. Front Immunol. 2025 Jun 25;16:1597736. doi: 10.3389/fimmu.2025.1597736.

Recent Literature on MAP (continued)

Zapico D, Espinosa J, Muñoz M, Reyes LE, Benavides J, Marín JFG, Fernández M. Influence of Paratuberculosis Vaccination on the Local Immune Response in Experimentally Infected Calves: An Immunohistochemical Analysis. Animals (Basel). 2025 Jun 22;15(13):1841. doi: 10.3390/ani15131841.

Singh VK, Jatav GP, Singh R, Gangwar N, Das C, Yadav SK. <u>Histo-Pathological and Serological Investigation on the Occurrence of Subclinical **Paratuberculosis** in Slaughtered Buffaloes in Mhow, <u>Madhya Pradesh, India.</u> Indian J Microbiol. 2025 Jun;65(2):979-990. doi: 10.1007/s12088-024-01360-0.</u>

Tabrizi EF, Zolfaghari MR, Mosavari N, Nazari R, Tadayon K. <u>New mini-satellite tandem repeat markers for strain typing of *Mycobacterium avium* subsp. *paratuberculosis*. Mol Biol Rep. 2025 Jul 14;52(1):709. doi: 10.1007/s11033-025-10792-w.</u>

Altaf M, Farooq S, Bhat MA, Kashoo ZA, Yangzom P, Tramboo U, Qureshi S, Ul-Hussain I, Allaie IM. Serological detection and molecular typing of *Mycobacterium avium* subspecies *paratuberculosis* in sheep and goats co-infected with gastrointestinal parasites from the northern Himalayan territories of India. J Appl Microbiol. 2025 Jul 1;136(7):lxaf180. doi: 10.1093/jambio/lxaf180.

Singh VK, Gupta V, Das C, Kumar A, Yadav SK. <u>Polymerase spiral reaction assay for rapid visual detection of *Mycobacterium avium* subsp. *paratuberculosis* in fecal samples. Sci Rep. 2025 Jul 25;15(1):27149. doi: 10.1038/s41598-025-12435-3.</u>

Idris SM, Elmagzoub WA, Okuni JB, Ojok L, Mukhtar ME, Abdalla EM, El Sanousi SM, Amanzada A, Truyen U, El Wahed AA, Eltayeb E, Eltom KH, Gameel AA. <u>Paratuberculosis in small ruminants in the Sudan: prevalence and risk factors.</u> BMC Vet Res. 2025 Jul 29;21(1):496. doi: 10.1186/s12917-025-04920-8.

Barth SA, Peters M, Mormann S, Möbius P, Calvelage S, Brangsch H. <u>First whole-genome sequence</u> of *Mycobacterium avium* subsp. *silvaticum* isolated from a diseased Egyptian goose (*Alopochen aegyptiaca*). BMC Genomics. 2025 Aug 11;26(1):741. doi: 10.1186/s12864-025-11893-3.

Garbarino C, Nava M, Filippi A, Forti A, Russo S, Barsi F, Bianchi A, Filipe J, Arrigoni N, Pedrotti L, Corlatti L, Ricchi M, Luzzago C. Contamination of alpine pastures by *Mycobacterium avium* subsp. *paratuberculosis*: Evaluation of cattle and red deer contribution through environmental fecal samples. Vet Microbiol. 2025 Aug 5;309:110661. doi: 10.1016/j.vetmic.2025.110661.

Marcelino I, Keizer J, Monti G, Cornelissen P, Santman-Berends I, Lam JH, van der Poel WHM. Monitoring Pathogens in Free-Living Large Herbivores in a Nature Reserve in the Netherlands. Transbound Emerg Dis. 2025 Aug 8;2025:6948049. doi: 10.1155/tbed/6948049.

Ulloa F, Penati M, Hernández-Agudelo JM, Tejeda C, Steuer P, Addis MF, Mella A, Canales N, Soto JP, Salgado M. Analysis of pathogenic bacteria and antimicrobial residues in bovine waste milk on dairy farms in southern Chile. Front Vet Sci. 2025 Aug 1;12:1613185. doi: 10.3389/fvets.2025.1613185.

Rashidifar S, Harzandi N, Gharavi MJ, Momeni Z, Nezhad MH. <u>Direct detection and rapid speciation</u> and subspeciation of *Mycobacterium avium* complex using five-target multiplex PCR and clinical correlations in HIV-positive patients in Tehran, Iran. Diagn Microbiol Infect Dis. 2025 Aug 12;113(4):117064. doi: 10.1016/j.diagmicrobio.2025.117064.

Recent Literature on MAP (continued)

Hernández-Agudelo JM, Verdugo C, Barkema HW, Steuer P, Tejeda C, Ulloa F, Soto JP, Salgado MA. Early-life shedding and environmental presence of Mycobacterium avium ssp. paratuberculosis in Chilean dairy calves. J Dairy Sci. 2025 Aug 20:S0022-0302(25)00689-7. doi: 10.3168/jds.2025-26872.

Alonso-García M, Liaubet L, Faraut T, Dehais P, Kühn C, Demars J, Gutiérrez-Gil B, Robic A. Decoupling transcriptome layers: the distinct and variable nature of circular RNAs. BMC Biol. 2025 Aug 27;23(1):269. doi: 10.1186/s12915-025-02375-9.

Rashidifar S, Harzandi N, Gharavi MJ, Momeni Z, Nezhad MH. Direct detection and rapid speciation and subspeciation of Mycobacterium avium complex using five-target multiplex PCR and clinical correlations in HIV-positive patients in Tehran, Iran, Diagn Microbiol Infect Dis. 2025 Dec;113(4):117064. doi:10.1016/j.diagmicrobio.2025.117064

Hananeh W, Fraiwan M. A histopathology image dataset for Johne's disease detection and research. Data Brief. 2025 Aug 12;62:111975. doi: 10.1016/j.dib.2025.111975.

Agrawal S, Gupta S, Singh SV. Exploring the role of circulating microRNAs as potential diagnostic markers in Mycobacterium paratuberculosis. Vet Ital. 2025 Sep 2;61(3). doi: 10.12834/Vetlt.3673.34650.3.

Venkatesh KM, Lopez-Villalobos N, Gupta SK, Udy GB, Laven R, Chiu SJ, Bugde P, Furuya Y, Dukkipati VSR. A comparative study between milk- and serum-based antibody detection assays for Johne's disease in New Zealand dairy cattle. Tuberculosis (Edinb). 2025 Aug 27:102679. doi: 10.1016/j.tube.2025.102679.

Griss S, Schüpbach-Regula G, Carmo LP, Meylan M, Thomann B. Economic viability of different surveillance strategies for the control of paratuberculosis in Swiss dairy cattle. Prev Vet Med. 2025

Sep 4;245:106685. doi: 10.1016/j.prevetmed.2025.106685.

Lee HG, Bok EY, Seo SY, Cho A, Jung Y, Kang S, Hur TY, Oh SI. Evidence of in-utero Mycobacterium avium subsp. paratuberculosis infection in two pregnant goats: PCR detection, bacterial isolation, and molecular genotyping. J Vet Sci. 2025 Aug 6. doi: 10.4142/jvs.25029.

Baghbadorani MK, Lotfollahzadeh S, Hasanvand AK, Khabazan MH, Darabi BH. Prevalence of paratuberculosis in Murciano-Granadina dairy goats in Iran: seroprevalence in two large industrial herds and consequences. Vet Res Commun. 2025 Sep 12;49(6):309. doi: 10.1007/s11259-025-10869-8.

Lee JH, Lee E-S, Kyung SM, Xiang X-R, Park H-E, Shin M-K, Yoo HS. Functional analysis of the intracellular survival of Mycobacterium avium subsp. paratuberculosis in THP-1 cells using CRISPR interference. J Bacteriol. 2025 Sep 12:e0024425. doi: 10.1128/jb.00244-25.

Galgano M, Messina D, D'Amico F, Sposato A, Razzuoli E, Mrenoshki D, Trisolini C, Caldarola G, Beverelli M, Ridolfi D, Bulzacchelli C, Giannico A, Parisi A, Tateo A, Pratelli A, Buonavoglia D. Seroprevalence study of paratuberculosis: Johne's disease, a neglected infection in dairy herds in Apulia (southern Italy). Vet Rec Open. 2025 Sep 11;12(2):e70020. doi: 10.1002/vro2.70020.

Dixit M, Filia G, Singh SV, Raies Ul Islam M. IS1311 PCR-RFLP molecular epidemiological approach for genotyping of Mycobacterium avium subspecies paratuberculosis in sheep and goat milk. Iran J 20 Vet Res. 2025;26(1):1-7. doi: 10.22099/ijvr.2025.49432.7258