The Paratuberculosis Newsletter

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The official publication of the International Association for Paratuberculosis

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Note from the Editor

It is only a few months since the last ICP but preparations for our next meeting, the 15th ICP, are well on the way . Read on to find out our new President's vision for the IAP, results of the recent **newsletter survey and a research article describing sample error in the quantitative assessment of MAP within fecal specimens.**

Kumi de Silva

IAP business

Message from the President

Dear Colleagues,

I am ever so grateful to have been given the opportunity and your trust to serve as the 4th President of the IAP.

It is a privilege and honor to serve our community, and I look forward to working closely with the executive team, the Board, and each of the members and stakeholder groups of to help realize the full potential of the IAP.

Please join me in conveying deep thanks to Ramon Juste for his service and stewardship of the IAP over the past many years. Ramon will always be "El Presidente" to me, and working closely with the IAP executive team of Secretary Ray Sweeney, Vice-President Rod Chiodini and the Editor-in-Chief, Kumudika de Silva, we hope to build on the good work and the legacy that he, together with past presidents, Mike Collins and Rod Chiodini and their respective leadership teams, have left for the IAP.

The IAP appears to be at a key inflection point – and there is much to be done to achieve our shared vision, the purpose with which the IAP was founded, and for which we come together and remain as an association – "the advancement of knowledge and scientific achievement toward the eradication of paratuberculosis in domestic livestock and other species affected by *Mycobacterium avium* subsp. *paratuberculosis*".

So how do we plan to accomplish this? First by being transparent in our decision-making processes while ensuring accountability and a relentless focus on execution of mutually agreed upon goals and objectives.

Second, we must do this as well by building trust and being recognized as an honest broker and the go-to source for leadership and scientific evidence regarding all things paratuberculosis. This will require a willingness to debate serious issues, highlight key knowledge gaps, and do so with candor and civility, and we must look forward to making and seizing all opportunities to advance

the science and the awareness to achieving our vision of a paratuberculosis free world.

Finally, and perhaps most importantly, we can only achieve this through the enlightened engagement and support of all our key stakeholders – starting with the core of the Association – you – the member.

In closing, while the work to achieving our vision that remains before us is not easy, or may even be considered by some an impossible dream, we must persevere as it is a truly virtuous goal, and probably more relevant today than it has ever been since our founding, and I look forward to working closely with you in the coming years towards achieving this. I strongly believe that despite the seemingly daunting challenges we will face, our future is bright, and it is ours to make.

Thank you again for the privilege and honor to serve you and the IAP, as always, please feel free to reach out to me or anyone in our leadership team if there is anything that we may do to serve you and our Association better.

With kind regards,

Vivek Kapur

Policy Changes

The IAP Board has approved changes to the Association's policies and the establishment of an **International Collaborative Research Grant Program (ICRGP). These changes have been posted on the IAP website: http://www.paratuberculosis.net/pdf/IAP_Policies-2018.pdf.**

Further information about the ICRGP will be available soon.

Minutes of the IAP General Meeting– Draft

These draft Minutes are subject to approval by vote at the next meeting.

14ICP Riviera Maya, Mexico June 7, 2018

1) **The meeting was called to order by President Ramon Juste at 17:00**
2) **The minutes from the previous meeting (2016) were approved without objection**
3) President's report
    a. **New Officers and Directors were acknowledged:**
        i. **President Vivek Kapur, Vice-President Rod Chiodini**
        ii. **Board of Directors: Gilberto Chavez-Gris (Mexico), Bryan Markey (Ireland), Ad Koets (Netherlands), Eiichi Momotani (new role, Japan Board representative), Raphael Guatteo (France)**
    b. **Departing Board members thanked:**
        i. **Victor Rutten (Netherlands), Christine Fourichon (France), Gregers Jungersen (Denmark), Peter Mullowney (Ireland).**
    c. **Membership Expansion Committee: Ramon submitted a request for volunteers and only one response was received—the committee was not appointed**

d. International Collaboration initiative—Rod Chiodini worked on this with Murray Hines. The idea for grants to foster international research collaborations was presented. The plan is to take up the proposal following the closing of the financial books on the 14ICP to evaluate what the Treasury can provide.

e. In memoriam: Marie Thorel was remembered for her contributions to the IAP and paratuberculosis research and her recent passing was noted.

f. Additional activities during the last 2 years included publication of the Guidelines document and preparation for the 14ICP.

4) Secretary-Treasurer Report—Ray Sweeney

a. The IAP has once again been incorporated as a Limited Liability not-for profit corporation in Arkansas, USU.

b. The IAP now has 167 members from 32 countries. Membership numbers are very stable (12 ICP had 175, 13 ICP had 161).

c. Current Treasury balance is $63,515 which is also very stable over the last 3 ICP cycles.

5) Editor-in Chief report—Kumi de Silva

a. It has been difficult to obtain submissions for the Newsletter. Currently produced quarterly, discussion ensued about whether the Newsletter has outlived its usefulness. Kumi will poll the membership regarding the Newsletter to aid the Board in determining the future of the publication.

6) 13ICP report

a. Dr. Fourichon, chair of the LOC for Nantes was not in attendance. The Treasurer reported that the meeting was a financial success returning a profit to the ICP.

7) 14ICP report—Gilberto Chavez-Gris

a. The meeting budget is currently on track to return a small profit, approx. US$1000.

b. There are 160 registered delegates, 32 from Mexico and a 30% increase in registrations from Latin American countries.

8) 15ICP report—Bryan Markey

a. The meeting is scheduled for June 15-18, 2020 in Dublin Castle, Dublin, Ireland

b. The website is open and there is a link on the IAP website page.

9) 16ICP

a. The 16ICP will be held at Amity University, Jaipur, India and hosted by Drs. Jagdip Sohal Singh and G. F. Aseri.

10) Amendments to the ByLaws

a. Various amendments to the ByLaws were presented for approval by the membership. Each was approved in turn by vote of the Members present. These are included as an addendum to the minutes. [Secretary note: The amended ByLaws are now posted on the IAP website.]

11) Presentation of Awards:

a. **Helping Hands Awards were presented to: Ganesh Sonowane, Amit Singh, Mukta Jain, Jose Fernandez Agudelo and Isis Espeschit**
b. **Merkal Scholarships were presented to Lucy Luo and Caroline Corbett**
c. **Emeritus Awards were presented to Douwe Bakker and Murray Hines**

12) Remarks by Incoming President

a. **Vivek Kapur addressed the Membership**

13) Recognition of Ramon Juste

a. **Ramon Juste was recognized for his service to the IAP as a longstanding Board Member and President of the association and was presented with a commemorative plaque.**

14) Meeting was adjourned at 19:00

Respectfully Submitted

Raymond W. Sweeney, VMD

Secretary-Treasurer

Appendix I: Proposed by Laws Changes approved at General Membership meeting

Proposed changes to the Laws & By-Laws of the Association

ARTICLE I. MEMBERSHIP

Section 3. Dues.

Current language:

(b) The dues for any member shall be remitted or reduced by vote of the Governing Board

Proposed Change:

(b) The dues for any member ~~shall~~ may be remitted or reduced by vote of the Governing Board

Reason and Justification for proposed change:

The word "shall" is a mandated requirement and its use is inappropriate here.

Section 5. Resignation and Expulsions.

Current language:

(c) Any member whose dues are two years in arrears shall be dropped from the Association, but membership shall be reinstated if all arrears are made up before reinstatement.

Proposed Change:

(c) Any member whose dues are two years or more in arrears shall be dropped from the Association, but membership shall be reinstated if ~~all~~ arrears are made up before reinstatement, up to a maximum cost/fee equal to 1-year's dues.

Reason and Justification for proposed change:

Now that memberships are controlled and automatically calculated electronically, penalties for being in arrears are being automatically implemented. A recent member was required to pay 3-years membership ($140.00) for an approximate 6-month membership due to a 2-1/2 year lapse in dues. It was felt that this was an excessive "penalty". Although nothing could be done at that time, changing the Laws & By-Laws would reduce this penalty to a more reasonable level.

ARTICLE II. OFFICERS

Current language:

Section 5. Secretary-Treasurer.

…The accounts shall be audited annually or as necessary.

***Proposed Change*:**

…The accounts shall be audited ~~annually or~~ as deemed necessary by the Board of Directors.

Reason and Justification for proposed change:

To my knowledge, the Association accounts have never been audited and the Association has hence been in violation of its Laws & By-Laws. This change is needed to stop this continued violation. The Officers of the Association are excluded from involvement in the audit due to potential conflicts of interest.

ARTICLE VII. SCIENTIFIC MEETINGS (COLLOQUIA)

Current language:

Section 1. Regular Meeting.

A meeting of the Association shall be held at least every 3 years at a place and time to be determined by the Governing Board. This shall include a General Membership Session for the conduct of business of the Association.

***Proposed Change*:**

A meeting of the Association shall be held at least every 2-3 years at a place and time to be determined by the Governing Board. This shall include a General Membership Session for the conduct of business of the Association.

Reason and Justification for proposed change:

Minor change but requested only because a general request to change the Laws & By-laws is being made and more accurately reflects the current frequency while maintaining flexibility.

ARTICLE VII. SCIENTIFIC MEETINGS (COLLOQUIA)

Current language:

Section 2. Name.

The scientific meeting of the Association shall be called the International Colloquium on Paratuberculosis and the first such meeting will bear the prefix 1st. Subsequent meetings will be numbered consecutively.

***Proposed Change*:**

The scientific meeting of the Association shall be called the International Colloquium on Paratuberculosis and the first such meeting will bear the prefix 3rd. Subsequent meetings will be numbered consecutively.

Reason and Justification for proposed change:

Someone erroneously changed this from the original Laws & By-Laws. The 1st and 2nd Colloquia were pre-Association and hence the 1st Colloquium of the Association bears the prefix 3rd.

Current language:

Section 4. Meetings of the Governing Board.

The Governing Board shall hold at least one business meeting during the general meeting of the Association, conducted in conformity with parliamentary procedure. Five members of the Governing Board shall constitute a quorum for the transaction of business. The act of the majority of the members of the Governing Board present at a meeting at which a quorum is present shall be the act of the Board. During periods between meetings of the Association, the Governing Board shall make decisions by written telecommunications. At least 5 Governing Board members must respond to constitute a quorum. The act of the majority of the members of the Governing Board shall be the act of the Governing Board. At least 5 days shall be provided for a reply by individual members of the Board of Directors.

***Proposed Change*:**

The Governing Board shall hold at least one Executive Meeting during the general meeting of the Association, conducted in conformity with parliamentary procedure. Five members of the Governing Board shall constitute a quorum for the transaction of business. The act of the majority of the members of the Governing Board present at a meeting at which a quorum is present shall be the act of the Board. During periods between meetings of the Association, the Governing Board shall make decisions by written telecommunications. At least 5 Governing Board members must respond to constitute a quorum. The act of the majority of the members of the Governing Board shall be the act of the Governing Board. At least 5 days shall be provided for a reply by individual members of the Board of Directors.

Any Association member wishing to bring new or old business matters before the Board of Directors shall submit this business in writing to his/her representative on the Board of Directors within the prescribed period before the meeting. If said member does not have a representative on the Board, matters shall be sent to the Secretary. Any Board Member wishing to bring new or old business matters before the Board of Directors Meeting shall submit this business in writing to the Secretary within the prescribed period before the meeting. The Board has the right, based on the act of the majority, to decide what business will be heard and discussed during the Executive Meeting. Board members shall be provided with a copy of the Meeting agenda, along with any supporting material, no later than 48 hours prior to said meeting. All actions of the Board must be approved by a simple majority of the General Membership before enactment or implementation.

Any member wishing to bring new or old business before the General Membership Session of the Association that has not been placed on the agenda by the Governing Board may do so under suspension of the rules. Rules of procedure may be suspended at any meeting by a vote of two-thirds of the membership present. However, rules may not be suspended for consideration of new or old business unless the President is notified 24 hours before the beginning of the meeting that such a request is to be made and the membership is notified early in the meeting of the nature of the new or old business to be discussed under suspension of the rules if voted.

Reason and Justification for proposed change:

This language is very similar to that under the General Association Meeting. At present, members of the Association only have a mechanism to bring business up at the general Association Membership meeting without any defined mechanism to address the Board of Directors. Thus, the first clause provides such a mechanism. There also does not exist a formal or defined mechanism for Board Members themselves to officially submit business items to the Executive Meeting. Without any "prep-time" of the Board Members or full knowledge with supporting materials that will be discussed, these meetings tend to unnecessarily drag on. These amendments/changes are made to facilitate and expedite these meetings. Finally the Board should not have the authority to enact policies or otherwise take actions that may affect the membership without giving the membership the ability to approve or disapprove.

ARTICLE X. COMMITTEES

Current language:

The Governing Board may from time to time designate ad hoc committees to consider matters of interest to the Association. The number of members, the designation of the chairperson, the

terms of membership, and the duration of the duties of each such ad hoc committee shall be determined by the Governing Board.

***Proposed Change*:**

The Governing Board may from time to time designate ad hoc committees to consider matters of interest to the Association. The number of members, the designation of the chairperson, the terms of membership, and the duration of the duties of each such ad hoc committee shall be determined by the Governing Board. Members of ad hoc committees must be members of the Association and recommendations made by these committees must receive Board and Membership approval before implementation.

Reason and Justification for proposed change:

"Matters of interest to the Association" should not be considered by non-members of the Association and implementation of recommendations should not be decided exclusively by the Board without approval by the membership at large.

15th ICP

The next International Colloquium on Paratuberculosis will be held in Dublin on June 13th - 18th 2020. We have selected Dublin Castle as the conference venue in the centre of Dublin. We have set up a website at https://www.icpdublin.com/ which will be continually updated. Twelve people have agreed to be on the Local Organising Committee and we will be meeting shortly to assign roles for the Scientific Committee. The main topics to be covered will be the same as previous colloquia namely:

1. Diagnostics and detection
2. Host response and immunology
3. Control programs
4. Pathogenomics and Map biology
5. Genotyping and Map diversity
6. Epidemiology
7. Public health and Map in the environment.

We are going to have a page on the website with links to all the articles published on Johne's Disease in the last five years and will invite all the authors to submit an abstract for presentation in Dublin in two years' time. We will be submitting an item to each of the forthcoming Paratuberculosis Newsletters to keep you informed of progress in the organisation of ICP 2020.

Peter Mullowney

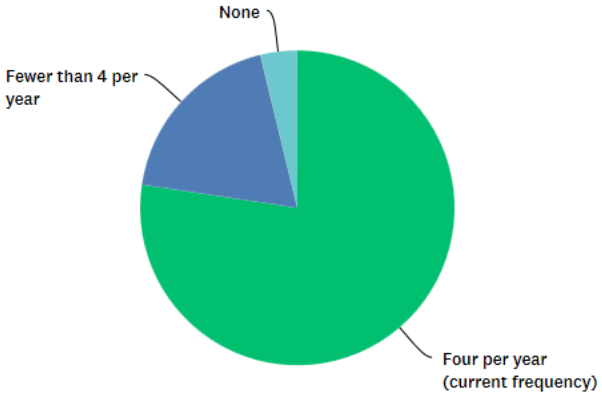
Equity and Access Policy

The policy has been drafted and will be submitted to the IAP Board for discussion.

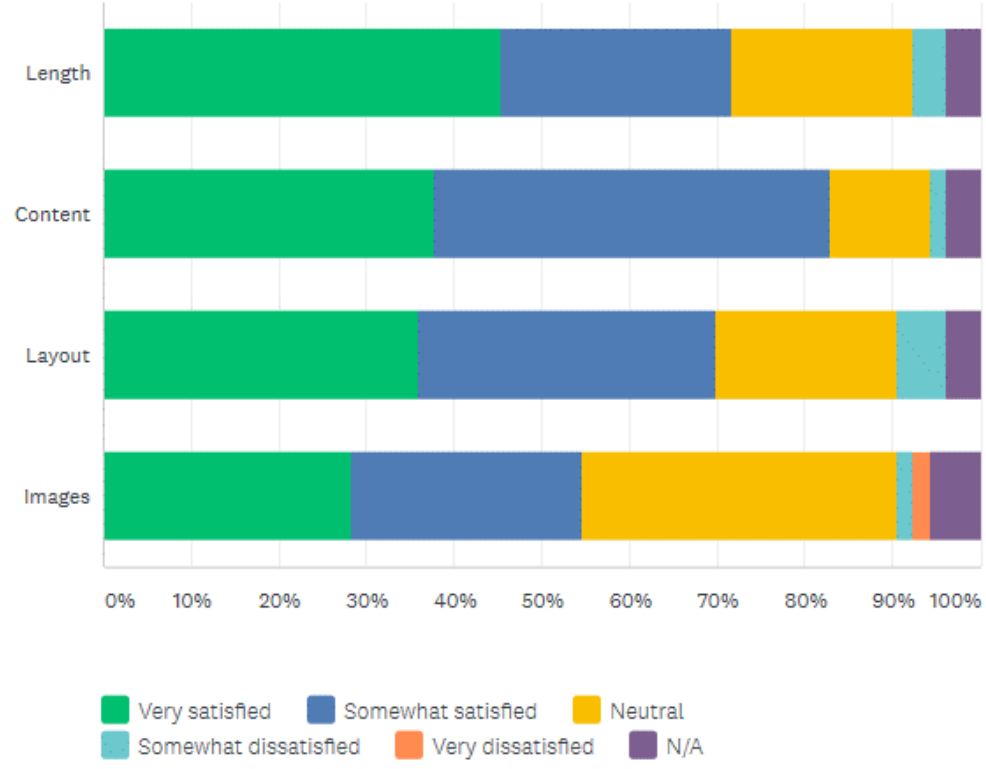
Newsletter survey

In August all current IAP members were sent a request to participate in a survey about the Newsletter. The response rate was 31%.

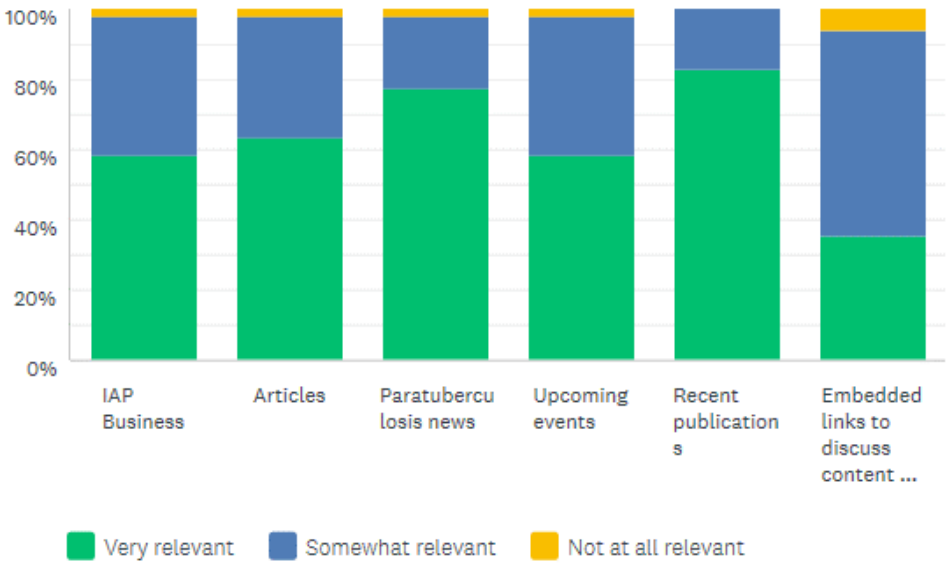
Most responders read the Newsletter (every issue 55%, some issues 42%) and 80% had read one or more of the Newsletters this year. "Being too busy" was the main reason for responders not reading the Newsletter and accessing the Newsletter was a problem for a few. Most were happy with the current frequency of the Newsletter



The majority (85%) were very satisfied/satisfied with the current newsletter. Unsurprising given that these IAP members were motivated enough to take the survey! Other details of what the responders liked or disliked are shown in the following figures.



Responder satisfaction with the current Newsletter



Responder satisfaction with Newsletter content

To further enhance the Newsletter, responders mainly requested content related to research groups and regional news about paratuberculosis. Other comments were to include technical tips and job opportunities related to paratuberculosis, to revert to the previous layout for the newsletter, to make the Newsletter more modern and that the Newsletter was still an important means of communication for the IAP.

Thank you to everyone who took the time to complete the survey. This information will be used to maintain a newsletter that is useful and relevant to your needs.

Job Opportunities

University of Liverpool, UK

Chair/Reader/Senior Lecturer in Infection Immunology Grade 9/10

Application Deadline: 31-Oct-2018 23:30

Sample error in the quantitative assessment of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) within fecal specimens

Gilles R. G. Monif [1,2], J. Elliot Williams[2], Tseng Long Lin[3], Ching Ching Wu[3]

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[2]University of Florida College of Veterinary Medicine, Gainesville, FL 32611
[3]Purdue University School of Veterinary Medicine, West Lafayette, IN 4790

Abstract

Because *Mycobacterium avium* subspecies *paratuberculosis* (MAP) tends to grow in tight clumps of varying sizes, a comparative, prospective, blinded study was done to determine whether the magnitude of mycobacterium assessment by culture could be substantiated using PCR technology. Test samples derived from the same fecal specimen were assessed by Trek® Diagnostic System, hspX PCR, and direct and nested IS1311-based PCR. Forty-five percent of fecal samples that had heavy presence of MAP and 70% of fecal samples that had moderate presence of MAP did not have MAP DNA identified in separate fecal samples taken from the same fecal specimen. Only 3 out of 43 cows designated as having significant fecal shedding were demonstrated to have anti-Map antibodies. The lack of concurrence between the significant fecal presence of MAP by culture and two sensitive PCR tests, coupled with the absence of serological evidence of MAP infection in the majority of cases, argues that sample error, compounded by probable variability in MAP's manner of growth, can cause quantitative misrepresentation of the amount of MAP present and misrepresent the animals underlying clinical status.

Introduction

A major herd management tool in controlling Johne's disease within production animal groupings has been the ability to quantify the amount of MAP present in a given fecal specimen (Collins). Animals that demonstrate heavy MAP fecal shedding are considered to represent a greater threat to overall herd health and are frequently culled.

Mycobacterium avium subspecies *paratuberculosis* (MAP) differs from other pathogenic mycobacterium, such as *Mycobacterium bovis* and *Mycobacterium avium* subspecies *avium*, by its growth in tight clumps (Schleig, Hoal-Van Helden, Harris). Depending upon the portion of the specimen selected for testing within a given fecal specimen, sample error and over representation of MAP due to variation in clump size can theoretically introduce the potential for significant sampling error. No studies have been conducted to analyze whether sample site bias occurs.

The purpose of this paper is to report PCR results derived from two separate samples taken from the same fecal specimen that the Trek® Diagnostic System identified as having a significant number of MAP colonies.

Materials & Methods

Study Material: In a collaborative arrangement between the Florida USDA group and the Veterinary Diagnostic Laboratory at the University of Florida College of Veterinary Medicine, 347 fecal samples were obtained

from two dairy herds that participated in USDA's Florida Johne's Disease Dairy Herd Demonstration Project. The samples were shipped via Federal Express next day shipment in coolers with ice packs to the Animal Disease Diagnostic Laboratory, School of Veterinary Medicine at Purdue University and to the Veterinary Diagnostic Laboratory at the University of Florida College of Veterinary Medicine. Corresponding serum samples were sent directly to the State of Florida Veterinary Diagnostic Laboratory.

Study Population: The study population was determined by 22 fecal samples that had heavy mycobacterial growth identified and 22 fecal samples demonstrating moderate growth as determined by Trek® Diagnostic System. The culture determination of MAP was done at Animal Disease Diagnostic Laboratory, School of Veterinary Medicine at Purdue University, a USDA certified diagnostic facility for both the Trek® Diagnostic System and Tetracore® MAP Diagnostic System.

Serum Map ELISA Test: The serum Map ELISA testing (ParaChek® Prionic, Switzerland) was done at the State of Florida Veterinary Diagnostic Laboratory at Live Oak, Florida in accordance with that laboratory's established protocols. The State of Florida Veterinary Diagnostic Laboratory is fully certified by USDA in its performance of its MAP ELISA test.

HspX PCR Test: The hspX PCR data was used as furnished with the data point being whether the test was determined by laboratory criteria that demonstrate the presence or absence of MAP heat shock protein. The Animal Disease Diagnostic Laboratory at Purdue University is a USDA certified diagnostic facility for the Tetracore® MAP Diagnostic System.

IS1311 PCR Testing: The fecal samples shipped to the Veterinary Diagnostic Laboratory of the Department of Infectious Diseases, College of Veterinary Medicine were analyzed using direct and nested polymerase chain reaction primer pairs. After removal of PCR inhibitor and DNA extraction, the samples were probed with two pairs of primer: IS1-IS2 nested primers IS3 -IS4. Both pairs of primers are based upon the IS1311 insertion sequence. Primers IS1-IS2 (CGA TTT ATC AGG CAC TCA TCG/CAA ATA GGC CTC CAJ CAC CA) recognize a 242 base pair sequence of Map IS1311 and primers IS3-IS4 (ATG AAC GGA GCG CAT CAC /CGA CCG AAG CTT GGG AAT) overlap and span a 104 base pair region within the insertion sequence. The IS1311 primer pairs identify 6-8 copies. Positive and negative fecal controls were used in each test. Using the IS1-4 primers, Veterinary Diagnostic Laboratory of the Department of Infectious Diseases, College of Veterinary Medicine was certified in the USDA MAP Fecal Laboratory Certification Test.

Data Computation: The test results from all three veterinary diagnostic laboratories were sent, as developed, directly to the USDA Office in Gainesville, Florida. In keeping with the experimental design, the results from the USDA's contracted laboratories were forwarded to Infectious Diseases Incorporated for secondary analysis. The test results from the Animal Disease Diagnostic Laboratory, School of Veterinary Medicine at Purdue University, the Veterinary Diagnostic Laboratory of the Department of Infectious Diseases at the University of Florida College of Veterinary Medicine and the State of Florida Veterinary Diagnostic Laboratory for the 22 alleged heavy shedders and the 21 moderate Map fecal shedders were recorded on a spreadsheet.

Statistical Analysis: Results for the hspX PCR and the IS1311-based PCR were analyzed to establish associations between the possible combinations of test pairs. Cohen's kappa coefficient was used as a measure of agreement between each pair of tests. The following ranges were considered for interpretation of the kappa coefficient (Landis and Koch, 1977); poor agreement: <0.00; slight agreement: 0.00–0.20; fair agreement: 0.21–0.40; moderate agreement: 0.41–0.60; substantial agreement: 0.61–0.80; almost perfect: 0.81–1.00.

The association between the levels of shedding (high vs. moderate shedding) and the probability of a positive test result was analyzed by logistic regression. Data were analyzed using the SAS statistical package for Windows (SAS Systems for Windows Version 9.00; SAS Institute Inc., Cary, NC, USA) using the PROC FREQ and PROC GLIMMIX. Values of $P \leq 0.05$ were considered significant for all tests.

Results

PCR Tests: Both the hspX and the IS1311 nested PCR tests identified the presence of MAP DNA in 7 of the 22 designated heavy shedders. In five other specimens, MAP DNA was identified by either the hspX or IS1311 PCR tests. Ten fecal specimens (45%) did not contain MAP DNA detectable by either by hspX or nested IS1311 PCR tests done on samples derived from the same fecal submission analyzed by the Trek® Diagnostic System.

Twenty-one other fecal specimens were identified as demonstrating the moderate presence of MAP in their fecal sample. In only two fecal samples, the presence of MAP DNA was documented by both PCR tests. Four other specimens had the presence of MAP DNA identified by either a positive real time or nested PCR test. For 15 (70%) fecal samples demonstrating moderate MAP fecal growth, neither the sensitive hspX nor IS1311 PCR test could demonstrate the presence of MAP DNA in the samples.

Map ELISA Test: In the heavy shedder group, the ParaChek ® Map ELISA test identified two sera as being MAP positive and one additional serum as having a high suspicious titer. When tested 14 months later, the cow with the suspicious titer had no demonstrable MAP antibodies. The other 18 sera tested MAP negative. Of the seven cows for which additional sera were tested 14 months later, none of these subsequent serum samples did not demonstrate the presence of anti-MAP antibodies.

In December 2006, the State of Florida Veterinary Laboratory reported that all 21 sera from the "moderate shedders" had tested negative. In May of 2007, one cow seroconverted. When the animals were retested in February 2008, the remaining 10 cows tested MAP negative.

Statistical Analysis: Kappa coefficients (95% CI) for the agreement of results were 0.43 (0.16-0.71) indicating a moderate level of agreement. The odds of a positive result for the hspX PCR in high shedder cows were 2.22 times the odds of a positive result in moderated shedders. Similarly, the odds of a positive result for the IS1311-based PCR in high shedder cows were 3.11 times the odds of a positive result in moderate shedders. However, in both cases the P-values were greater than 0.05 (0.21 and 0.10, respectively).

Discussion

Non-confirmation of positive culture identification of MAP by PCR in bovine fecal samples has been previously described. Soumya et al. retested 12 bovine fecal-culture positive samples that gave negative PCR results in nested PCR testing. Eight of the twelve fecal samples were positive in the new tests. Retesting had required resampling of the fecal sample. The current study data focuses the potential for contradicting test results between culture and PCR identification of MAP in bovine feces due to sample error.

When an animal is showing to have a large amount of MAP in its feces, the term "heavy shedder" has been used with the inference being that the animal has a more advanced gastrointestinal infection/disease status. The study data questions the assumption that quantitative assessment of MAP shedding alone can be a useful predictor of the data's significance. That 18 of the 21 sera available for testing failed to test MAP ELISA positive and when retested 14 months later, 16 of the remaining 18 cows still were serologically negative puts into question the use of quantitative data in making herd management decisions. This lack of a detectable serological response over time argues against the corresponding fecal samples specimens having come from an animal with advanced MAP infection.

The importance of correctly identifying heavy fecal shedding of MAP is two-fold: 1) animals identified as heavy shedders are considered to be primary disseminators of pathogenic mycobacterium into the environment; and 2) true heavy MAP replication in feces usually correlates with advanced gastrointestinal infection and increased probability of systemic progression.

The lack of concurrence between a culture indicative of heavy or moderate fecal MAP shedding and two sensitive PCR tests coupled with the failure of the vast majority of alleged heavy shedders to subsequently develop serological evidence of MAP infection demonstrates that significant interpretation error can occur due to sample error and probable variability in MAP's growth in clumps of varying size. The significance of "heavy shedding", as identified by the current culture technology, can be colored by definitely sample bias.

Conclusion

Sample error coupled with variability in the degree to which clumping has occurred can result in an erroneous perception of the magnitude of clinical involvement or stage of infection v. disease. In samples taken from the same fecal submission, 45% of fecal samples that had heavy presence of MAP and 70% of fecal samples that had moderate presence of MAP did not contain detectable Map DNA by two separate PCR technologies used. Of the 43 fecal cultures designated as demonstrating heavy shedding for which serological test results were available, only three had confirming diagnostic MAP antibody titers identified by the ParaChek® MAP ELISA test.

Decision makers may be well advised to seek additional confirmation before culling an animal from the herd based upon quantitative culture assessment.

References

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5. **Soumya M. P., Pillai R. M., Anthony P.X., Mukhopadhyay H. K., Rao V. N.**. 2009. Comparison of fecal cultures and IS900 PCR assay for the detection of *Mycobacterium avium* subspecies *paratuberculosis* in bovine fecal samples, Vet. Res, Commun. 33:78791

Acknowledgements:

The authors acknowledge the gracious collaboration and support received from the Florida Department of Agriculture and Consumer Services and United States Department of Agriculture- Florida.

Upcoming events

- **15th ICP on 13-18 June 2020 in Dublin, Ireland**

- **7th International Conference on *Mycobacterium bovis***

- **International Veterinary Immunology Symposium 2019 in Seattle, USA**

- **16th ICP in 2022 Jaipur, India**

Paratuberculosis News

The presence of viable MAP in pasteurized milk is in the spotlight again.

An article in the media on spring cleaning lists 'paratuberculosis' as one of the 'nasties' we need to get rid of in our homes

Recent publications (July-Sept 2018)

Begg, D. J., K. de Silva, L. Di Fiore, D. L. Taylor, K. Bower, L. Zhong, S. Kawaji, D. Emery and R. J. Whittington (2018). Corrigendum to Experimental infection model for Johne's disease using a lyophilised, pure culture, seedstock of Mycobacterium avium subspecies paratuberculosis [Veterinary Microbiology 141 (2010) 301-311]. Vet Microbiol.

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Corbett, C. S., J. De Buck and H. W. Barkema (2018). Effects of freezing on ability to detect Mycobacterium avium subsp. paratuberculosis from bovine tissues following culture. J Vet Diagn Invest 30(5): 743-746.

Corbett, C. S., S. A. Naqvi, J. De Buck, U. Kanevets, J. P. Kastelic and H. W. Barkema (2018). Environmental sample characteristics and herd size associated with decreased herd-level prevalence of Mycobacterium avium ssp. paratuberculosis. J Dairy Sci 101(9): 8092-8099.

de Souza, G. D. S., A. B. F. Rodriguez, M. I. Romano, E. S. Ribeiro, W. M. R. Oelemann, D. G. da Rocha, W. D. da Silva and E. B. Lasunskaia (2018). Identification of the Apa protein secreted by Mycobacterium avium subsp. paratuberculosis as a novel fecal biomarker for Johne's disease in cattle. Pathog Dis 76(6).

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Kim, W. S., M. K. Shin and S. J. Shin (2018). MAP1981c, a Putative Nucleic Acid-Binding Protein, Produced by Mycobacterium avium subsp. paratuberculosis, Induces Maturation of Dendritic Cells and Th1-Polarization. Front Cell Infect Microbiol 8: 206.

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All contributions should be sent to editor@paratuberculosis.net

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