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**Epidemiology of *Mycobacterium avium* subspecies  
*paratuberculosis* infection on sheep, beef cattle and deer  
farms in New Zealand**

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## Summary

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Paratuberculosis (Ptb) is a chronic enteric infection caused by *Mycobacterium avium* subspecies paratuberculosis (MAP), affecting wild and domestic ruminants. In domestic ruminants MAP infection is largely sub-clinical, but can result in chronic diarrhoea leading to emaciation and death. Clinical disease is commonly observed in adult cattle and sheep but in deer the disease incidence is higher in young animals (8-12 months). In the New Zealand pastoral farming system, it is common practice to co-graze Ptb susceptible livestock species (sheep, cattle, and deer) together, either concurrently or successively, on the same pasture. Thus several susceptible species have contact at farm level, being at risk of transmitting MAP between species through contaminated pasture. Johnes' Disease Research Consortium (JDRC), a partnership between livestock industries, government and research providers was created to study Ptb in an overarching approach, involving all susceptible species, aiming to generate scientific knowledge to support Ptb control policies.

The present research was implemented under the financial support of JDRC, aiming to generate epidemiological information about Ptb infection and clinical disease on mixed-species pastoral farms, grazing sheep, beef cattle, and/or deer. A total of 350 mixed-species farms (11,089 animals) were faecal and blood sampled and related epidemiological information was collected. Data was used to estimate: i) the national herd level true prevalence (HTP) of MAP infection on sheep, beef cattle and deer, ii) the risk of MAP infection and clinical disease incidence associated with species co-grazing,

iii) the association between infected and affected herds/flocks and production outputs, and iv) relationships between molecular strain types of MAP isolates and their distribution across livestock sectors and geographical areas. Finally, data and results from previous studies allowed v) the development and calibration of a two host-species (sheep & beef cattle) mathematical model, simulating MAP transmission between species and the effect of several control measures under mixed species farming.

MAP infection is widely spread in New Zealand. A Bayesian analysis to account for lack of sensitivity (Se) and specificity (Sp) of testing protocols, indicated that the highest HTP estimate for sheep flocks (75%, posterior probability interval (PPI) 68-82%), followed by deer (46%, PPI 39-54%) and beef herds (43%, PPI 35-51%). Sheep and beef cattle flocks/herds presented a higher prevalence in the North Island (NI), whereas deer infection was mainly located in the South Island (SI).

Logistic and Poisson regression models using Bayesian inference to adjust for lack of Se and Sp of diagnostic tests and of farmer's recall of clinical Ptb indicated that the shared use of pasture was associated with Ptb prevalence and incidence. When beef cattle and sheep were co-grazed, the infection risk increased 3-4 times in each species. Similarly, co-grazing of beef cattle and deer increased 3 times the risk of infection on deer. Co-grazing beef cattle with sheep, or beef cattle with deer, also was associated with increased clinical incidence in these species. Conversely, the co-grazing of sheep and deer was associated with a lower clinical disease incidence in both species.

Classical logistic and Poisson regression models indicated that MAP 'infection' status was significantly ( $p = 0.03$ ) associated with reduced calving rates in beef cattle herds and lower culling rates in deer herds and sheep flocks. Moreover, in sheep flocks and deer herds, a significant and a marginally significant ( $p = 0.05$  and  $0.09$ , respectively)

association were observed between ‘affected’ flocks/herds and lower tailing rates in sheep and weaning rates in deer, respectively.

Molecular analysis of MAP isolates obtained from sheep, cattle (beef and dairy) and deer, using a combination of the variable number of tandem repeats (VNTR) method and the short sequence repeat (SSR) method, rendered 17 MAP subtypes. Analysis indicated significantly higher subtype richness in dairy cattle and livestock sector as the main source of subtype variation. Moreover, similar subtypes were sourced from sheep and beef cattle, which tended to be different to the ones obtained from other livestock sectors. However, when beef cattle and deer were both present on the same farm, they harboured similar subtypes. These results provided strong evidence for transmission of MAP between species through the joint use of pasture.

Simulation results of a mathematical infectious disease model for Ptb indicated that the length of the co-grazing period was positively associated with the infection prevalence of sheep and beef cattle. Long pasture spelling periods from 9 to 15 months reduced MAP contamination up to 99%. However, the infection of naïve animals was still possible, but the prevalence remained <1% for at least 25 years. The simultaneous application of control measures on both species was the most efficient approach to reduce the prevalence and incidence. The separation of co-grazed species in tandem with an increased farmer surveillance, to reduce the time that clinical animals remained on the farm, was most effective in sheep, whereas T&C was in beef cattle.

The present research provides evidence that MAP infection is highly endemic in New Zealand farming livestock, and that the clinical disease incidence is generally low (<0.5%) in most infected farms. Moreover, inference from molecular pathogen typing of strategically collected isolates from farms across New Zealand strongly suggested that MAP is transmitted between species, mainly from sheep to beef cattle and between

beef cattle and deer, all of which are commonly grazed together in the New Zealand pastoral farming system.

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## Acknowledgements

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Probably this is the last part that everyone writes in their thesis. However, it is the most important part of the study because at this point you should have realized that carried out a PhD thesis; it is an impossible endeavour without the help and collaboration of other people. In my particular case, I have to say that the list of people that I have to give my most sincere thanks is quite long and without them, probably I would not be writing these words.

First, I have to acknowledge to my parents, without them I would not be here (technically) and this thesis would not have been written. I am not saying that this research would not have been done, I am pretty sure that there are several people more qualified and smarter than me, that would had been interested in take this challenge. However, it would not have been the same because this thesis reflects in certain way, the person that I am. And please do not think about the previous lines as a pointless exercise of self-adoration, rather my intention is totally the opposite. The person that I am is the outcome of all people that I have met through my life, and to all of you I am very thankful. You have made me a better person, I look back and I think that I have been lucky. During my PhD studies I have not just grown up as a professional or scientist, I have also grown up as a human being. Sorry if I am boring you but this is the only part (in more than 300 pages), where I have been allowed to divagate with my thoughts, so I am taking the opportunity.



Obviously, it is impossible to name all the people here, and I say sorry if I have omitted your name here. As I said before, my parents deserve a special thanks, despite of their humble origin they always teach me that I could be better person, that I could achieve any goal I propose and never (ever) give up. Paulina, probably the only person that really knows me, you have been my friend, my adventure partner, my wife and now the mother of my child. I am very thankful for met you, for all your support. Paulina, you are the perfect balance of my life. To my family also a very special thanks, independently that they never have understood what exactly I have been doing, they always have been there supporting and helping me.

Cord Heuer and Peter Wilson have been the cornerstone of this research, despite of that several times I felt compelled to throw the chair through the window after reading their comments of my papers. Always they have made very appropriate and assertive comments which have contributed to improve the present research and have helped me to be a better scientist. However, what I really want to acknowledge is our friendship. Independently that you always are going to be my professors, I consider you my friends. To Lesley and Anou, we shared the same tutors so in certain way our friendship was the closest that I have been of a therapy group. I really enjoyed our conversations about life, which were properly complemented with some (or several) bottles of wine. To Juan and Daniela, some of the nicer people that I have ever met, I really appreciate our long friendship and I hope the best for you guys. Nelly, one of the most tenacious person that I know, It was a pity that I could not stay for long in Palmy after your arrival, I am thankful of your friendship, always you have been very diligent with my research, even when you were on vacation with your family, you found some time to help me. Efrain, Andre, Philip, Kevin, and John, my partners on crime, I am going to remember you guys just with a big smile.

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Probably, at this point I got you totally bored, but in my defence I have to say that I trimmed the best jokes (self-censure) because they were just too politically un-correct. After this long acknowledgement section, I am pretty sure that you have understood why this thesis has more than 300 pages.



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## Nomenclature

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ACI	Annual clinical incidence
AFLP	Amplified fragment length polymorphism
AGID	Agar gel immunodiffusion
AMOVA	Analysis of molecular variance
AP	Apparent prevalence
AVLN	Abnormal visceral lymph nodes
BTM	Bulk tank milk
CD	Crohn's disease
CF	Complement fixation
CFU	Colony-forming unit
CG	Co-grazing
cPtb	Clinical paratuberculosis
DFA	Deer fenced area
DI	Diversity index
DIC	Deviance Information Criterion
Env	Simulation model environmental component
FC	Faecal culture
FTS	Farm type strata
GLM	Generalized Linear Models
HAP	Flock/herd level apparent prevalence

HPVj.neg	Joint herd-level predictive value negative
HPVj.pos	Joint herd-level predictive value positive
Hse	Herd-sensitivity
Hsej	Joint herd-level sensitivity
Hsp	Herd-specificity
Hspj	Joint herd-level specificity
HTP	Flock/herd-level true prevalence
IME	Individual milk ELISA
JDRC	Johne's Disease Research Consortium
LAM	Lipoarabinomannan
LIC	Livestock Improvement Corporation
MAC	Mycobacterium avium complex
MAP	Mycobacterium avium ssp. Paratuberculosis
MCMC	Markov Chain Monte Carlo
MPIL	Multiplex PCR of IS900 integration loci
NI	New Zealand North Island
OR	Odds Ratio
PCR	Polymerase chain reaction
PFC	Pooled faecal culture
PFGE	Pulsed field gel electrophoresis
POPR	Posterior probabilities
PPI	Posterior probability interval
PSI	Proportional similarity index
Ptb	Paratuberculosis
RFLP	Restriction fragment length polymorphism

RR	Relative risk
Se	Sensitivity
SI	New Zealand South Island
Sp	Specificity
SSR	Short sequence repeats
T&C	Test & cull
Th1	T-helper 1
TP	True prevalence
VNTR	Variable number of tandem repeats



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## List of Publications

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**Salgado M, Verdugo C, Castillo P, Zamorano P, Heuer C.** A novel low cost method for *Mycobacterium avium* subsp. *paratuberculosis* DNA extraction from an automated broth culture system for real time PCR confirmation. *Journal of Veterinary Science*, *accepted for publication*, 2013.

**Heuer C, Mitchell RM, Schukken YH, Lu Z, Verdugo C, Wilson PR.** Modelling transmission dynamics of paratuberculosis of red deer under pastoral farming conditions *PREVENTIVE VETERINARY MEDICINE*, Volume: 106, Issue: 1, Pp: 63-74, 2012.

**Stringer LA, Wilson PR, Heuer C, Hunnam JC, Verdugo C, Mackintosh CG.** Prevalence of *Mycobacterium avium* subsp. *paratuberculosis* in farmed red deer (*Cervus elaphus*) with grossly normal mesenteric lymph nodes. *New Zealand Veterinary Journal*, *Forthcoming articles*, Aug 2012.

**Dreyfus A, Verdugo C, Benschop J, Collins-Emerson J, Wilson P, Heuer C.** Leptospirosis sero-prevalence in New Zealand livestock and associated risk factors. *International Symposia on Veterinary Epidemiology and Economics proceedings*, *ISVEE13: Proceedings of the 13th International Symposium on Veterinary Epidemiology and Economics, Belgium, Netherlands*, Session 70 - One health, p 263, Aug 2012

**Marquetoux N, Mitchell R, Wilson P, Ridler A, Stevenson M, Verdugo C, Heuer C.** A within-herd state transmission model for MAP infection in farms co-grazing deer



and sheep in New Zealand. International Symposia on Veterinary Epidemiology and Economics proceedings, ISVEE13: *Proceedings of the 13th International Symposium on Veterinary Epidemiology and Economics, Belgium, Netherlands*, Poster topic 8 - Epidemiological and statistical tools and disease modeling, p 495, Aug 2012.

**Verdugo C, Mitchell R, Wilson P, Heuer C.** A two-strain mathematical model of paratuberculosis in a sheep-beef pastoral farming system in New Zealand. International Symposia on Veterinary Epidemiology and Economics proceedings, ISVEE13: *Proceedings of the 13th International Symposium on Veterinary Epidemiology and Economics, Belgium, Netherlands*, Poster topic 8 - Epidemiological and statistical tools and disease modeling, p 496, Aug 2012.

**Heuer C, Verdugo C, Price-Carter M, Collins D, French NP, Wilson PR.** Epidemiological inferences from typing *Mycobacterium avium* subsp. paratuberculosis (MAP) isolates from mixed sheep, deer and cattle farms. *Proceedings of the 11<sup>th</sup> International Colloquium on Paratuberculosis, Sydney, Australia*. 5 - 10 February 2012.

**Verdugo C, Jones G, Johnson W, Wilson P, Heuer C.** Bayesian latent class prevalence estimation of herd level infection with *Mycobacterium avium* subsp. Paratuberculosis. *Proceedings of the 11<sup>th</sup> International Colloquium on Paratuberculosis, Sydney, Australia*. 5 - 10 February 2012.

**Dreyfus A, Verdugo C, Benschop J, Collins-Emerson J, Wilson P, Heuer C.** Leptospirosis sero-prevalence and associated economic loss in New Zealand livestock. Proceedings of the Food Safety, Animal Welfare & Biosecurity, Epidemiology & Animal Health Management, and Industry branches of the NZVA, *Proceedings of the Epidemiology & Animal Health Management branch of the NZVA*. FCE Publication No. 291, p 3.12.1-3.12.10, Jul 2011.

**Verdugo C, Heuer C, Wilson P.** The association between Paratuberculosis and production performance in New Zealand pastoral livestock. Proceedings of the Food Safety, Animal Welfare & Biosecurity, Epidemiology & Animal Health Management, and Industry branches of the NZVA, *Proceedings of the Epidemiology & Animal Health Management branch of the NZVA*. FCE Publication No. 291, p 3.6.1-3.6.8, Jul 2011.

**Verdugo C, Heuer C, Prickaerts S, Wilson P.** Paratuberculosis prevalence in New Zealand pastoral livestock: a deer industry perspective. Proceedings of the Deer Branch of the New Zealand Veterinary Association, *Proceedings of the Deer Branch of the New Zealand Veterinary Association* - Volume 27, pp 35-38, Jan 2010.

**Dreyfus A, Verdugo C, Wilson PR, Benschop J, Collins-Emerson J, Heuer C.** A survey of clinical leptospirosis in animals and people on mixed-species farms: preliminary observations. Proceedings of the Deer Branch of the New Zealand Veterinary Association, *Proceedings of the Deer Branch of the New Zealand Veterinary Association* - Volume 27, pp 77-80, Jan 2010.

**Stringer LA, Wilson PR, Heuer C, Mackintosh CG, Glossop JC, Verdugo C.** Isolation of *Mycobacterium avium* subsp. Paratuberculosis from grossly normal mesenteric lymph nodes of slaughter deer, preliminary results from a nationwide prevalence study. Proceedings of the Deer Branch of the New Zealand Veterinary Association, *Proceedings of a Deer Course for Veterinarians* 2009, pp 67-71, Aug 2009.

**Verdugo C, Wilson P, Heuer C.** Association between species combination and grazing pattern on Johne's disease outbreaks and *Mycobacterium*-like lesions at slaughter in farmed deer. International Symposia on Veterinary Epidemiology and Economics proceedings, ISVEE 12: *Proceedings of the 12th Symposium of the International*

*Society for Veterinary Epidemiology and Economics, Durban, South Africa, p 101, Aug 2009.*

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## Preface

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“If everyone is thinking alike, then no one is thinking.”  
— Benjamin Franklin

“I can not change the world but I can change myself.”  
— Alejandro Jodorowsky

“- How long will take to learn this art?  
- A life, maybe a little more.”  
— Choi Hon Hi