P-047

Reduction in the severity of *Mycobacterium avium subsp. paratuberculosis* infection as calves age

Dairu Shu[1], Supatsak Subharat[1], D. Neil Wedlock[1], Geoffrey W. de Lisle[2] and Bryce M. Buddle[1]

[1]Hopkirk Research Institute, AgResearch, Palmerston North, New Zealand
[2]Wallaceville, AgResearch, Upper Hutt, New Zealand

Introduction:

Paratuberculosis is a chronic enteritis in ruminant, caused by *Mycobacterium avium subspecies paratuberculosis (MAP).* The cattle are generally infected at very young age but disease would not be diagnosed until late age. Within a short period after infection, a phase of multiplication of *MAP* occur in the walls of the small intestine. Whether MAPs are eliminated or the animal remains infected as latent carrier depending on the resistance of the individual. The proportion of animals in these categories is unknown. The mechanism of MAP elimination in young animals have not been well documented. Knowledge of immune response in cattle in early infectious period is crucial for early diagnosis and a strategic programme development for disease control.

Methods:

20 challenged MAP 10^9 bacilli orally, 3 times at weekly intervals

11 Non-challenge controls

Faecal samples at 2-3 month intervals for MAP culture. Blood samples periodically collected for monitor MAP-specific IFN-γ secretion and MAP-specific antibody

Euthanized and sampled at 7 or 15 month post challenge (PC)

Results:

MAP culture of faeces of MAP-challenged and control calves from 2 months to 15 months post-challenge (PC) and of tissue ileo-caecal valve (ICV), ileum, ileo-caecal lymph node (ICLN) and mesenteric lymph node (MLN) at 7 months and 15 months PC.

Group	MAP-challenged																				Control									
Animal no.	5	6	10	12	30	31	33	35	55	42	1	4	14	15	17	19	22	24	36	40	7	11	16	20	32	3	13	18	38	39	41
Month PC	Faecal MAP																														
2	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	-	+	+	-	-	-	-	-	NA	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+	-	+	-	NA	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	+	-	+	-	+	-	-	+	-	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
9											-	-	-	-	-	-	+	-	+	-									-	-	-
11	NA										-	-	-	-	-	+	+	-	+	-	NA								-	-	-
13											+	-	-	-	-	-	-	-	+	-									-	-	-
15											+	-	-	-	-	-	+	-	+	-									-	-	-
Tissure	7 month PC										15 month PC Tisure MAP										7 month PC					15 month PC				
IC	+	+	-	+	+	+	+	-	+	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ileum	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
ICLN	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
MLN	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-

+, positive; −, negative; NA, not available

Proportion of MAP positive tissue cultures for challenge calves at 7 months PC (36/40) was significantly greater than that for calves at 15 months PC (16/40), ($p < 0.05$)

Whole blood IFN-γ test and MAP specific antibody:
Proportions of positive from control and MAP-challenged calves from 3 months to 15 months PC

Group	Months post-challenge						
	3	6	7	9	11	13	15
	IFN-γ						
Control	0/10	0/11	0/11	0/6	0/6	0/6	0/6
MAP-challenged	7/20	19/20	18/20	7/10	8/10	9/10	7/10

All negative for serum antibody against MAP.

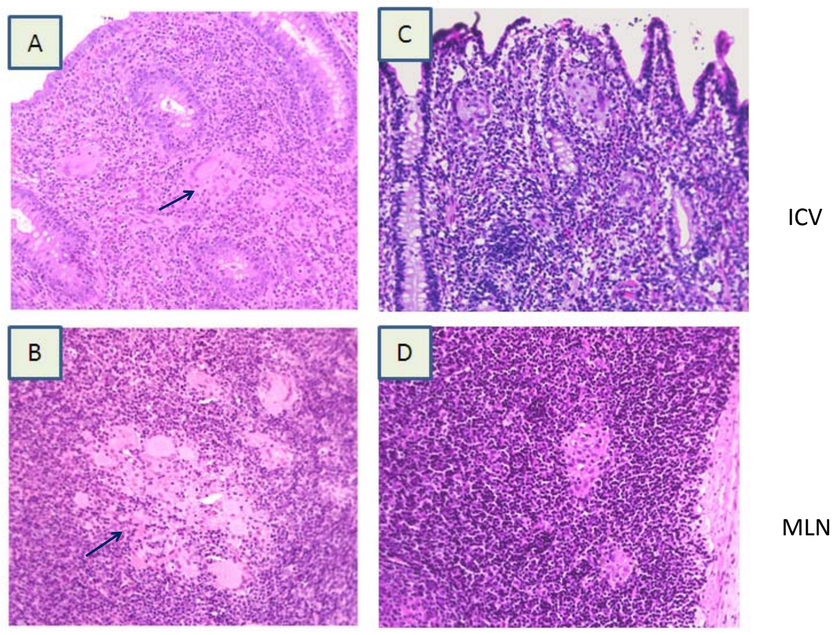
Histopathology

Histopathological scores of granulomatous lesions in the ileo-caecal valve (ICV), ileum, ileo-caecal lymph node (ICLN) and mesenteric lymph node (MLN) at 7 months and 15 months PC (0, no lesion; 1,scattered giant cells or small focal granuloma; 2, multifocal granuloma; 3, diffused granuloma; NA, not avalaible).

Group	MAP-challenged																				Control									
Animal no.	5	6	10	12	30	31	33	35	55	42	1	4	14	15	17	19	22	24	36	40	7	11	16	20	32	3	13	18	38	39	41
	7 month PC										15 month PC										7 month PC					15 month PC				
IC	0	1	0	1	1	1	0	1	0	2	0	0	0	0	0	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Ileum	0	1	0	0	0	2	0	1	0	1	1	0	0	0	NA	0	2	0	2	NA	0	0	0	0	0	0	0	0	0	0	0
ICLN	2	1	2	1	1	3	3	2	1	3	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
MLN	1	1	1	1	3	3	1	3	1	1	1	0	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Lesion scores /tissue for the challenged calves were significantly greater for those killed 7 months PC compared to that of 15 months PC ($p < 0.05$).

Histopathological lesions in MAP-challenged calves



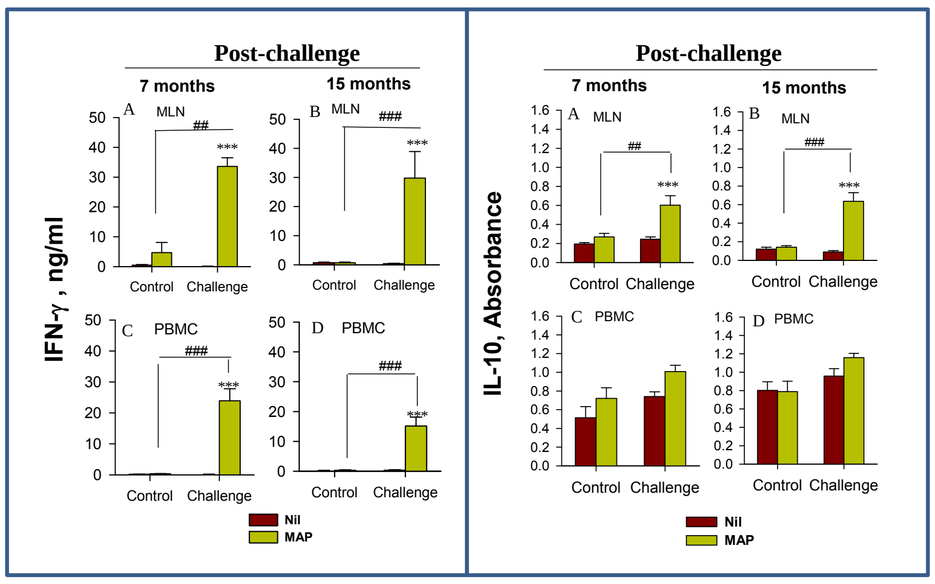
7 months PC
Multi focal granulomatous lesion with multinucleated giant cells (↑) present in ICV (A) and diffused granuloma (↑) in MLN (B)

15 months PC
Multifocal granuloma in ICV (C) less pronounced focal lesion in MLN (D)

Acid fast bacilli observed in sections of intestine and associated lymph nodes were sparse.

Secreted IFN-γ and IL-10:

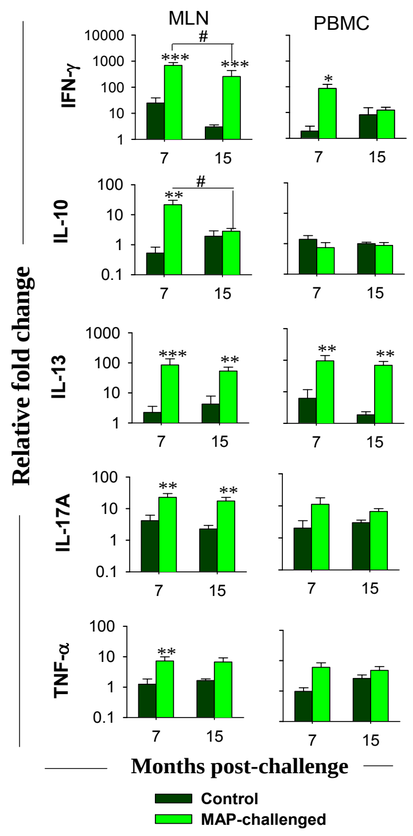
Secreted IFN-γ and IL-10 released in the cultures of MLN leukocytes and PBMCs from the control at 7 months (n=5) and 15 months (6) and MAP-challenged calves at 7 months (n=10) and 15 months (n=10) post-challenge. Culture cells were stimulated with MAP sonicate for 1 day compared to culture with media alone (nil). Data were presented as mean ± SE.



*: MAP sonicate-stimulation vs nil, ***P < 0.001.
#: MAP-challenged vs control calves, ##, P < 0.01; ###, P < 0.001

Cytokine mRNA expression

Cytokine mRNA expression for IFN-γ, IL-10, IL-13, IL-17A and TNF-α in cultures of MLN cells (A) and PBMCs (B) from 7 months post-challenged (control = 5, MAP-challenged = 10) and 15 months post-challenged (control = 6, MAP-challenged = 10). Cells were stimulated with MAP sonicate for 20 hours. The results were presented as mean relative fold change ± SE.



↑: Up-regulation ($P < 0.05$).
*: MAP-challenged vs control calves, *($P < 0.05$) and **($P < 0.01$).
#: 7 months PC vs 15 months PC ($P < 0.05$).

Summary:

➢MAP infection was established in the intestinal tract of all 20 challenged calves as indicated by culture of MAP from their faeces or tissues, while all the control calves were culture negative.

➢The proportion of challenged calves shedding MAP in their faeces decreased by 7 months PC. In contrast, the proportion of challenged calves positive for MAP-specific IFN-γ remained high from 6-7 months PC. None of the animals were positive for MAP serology.

➢Granulomatous microscopic lesions were present in all challenged calves by 7 months PC, but were less pronounced in those killed at 15 months PC.

➢mRNA expression of a diverse range of cytokines (IFN-γ, IL-10, IL-13, IL-17A and TNF-α) was up-regulated in MLN cell culture for calves killed at 7 and 15 months PC compared to the controls. However the up-regulation of IFN-γ and IL-10 from challenged calves was significantly less for those killed at 15 months PC compared to those at 7 months PC.

Conclusion

- Between 7 and 15 months PC there appeared to be partial control of MAP infection in the challenged calves.
- The partial control was associated with a significantly reduced up-regulation of gene expression of IFN-γ and IL-10 for the 15 months PC group compared to that for 7 months PC calves.

Acknowledgements

Tania Wilson, Allison McCarthy, and Gary Yates

Funding

New Zealand Johne's Disease Research Consortium
AgResearch / Moredun Postdoctoral Fellowship