

ANTEMORTEM DIAGNOSIS OF CAPRINE PARATUBERCULOSIS BY FAECAL PCR IN RELATION TO HISTOPATHOLOGICAL LESIONS

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Paratuberculosis (PTB) is a widely distributed disease caused by *Mycobacterium avium subsp. paratuberculosis* (Map). The antemortem diagnosis of Map-excreting animals is necessary to control PTB, especially in ruminants, hence a reliable PCR protocol for faeces would be useful.

OBJECTIVE

Determine the PTB prevalence in discarded goats and analyze the association between faecal-PCR and histopathological lesions.

MATERIALS AND METHODS

Necropsy

21 goats (> 3 years).



Samples

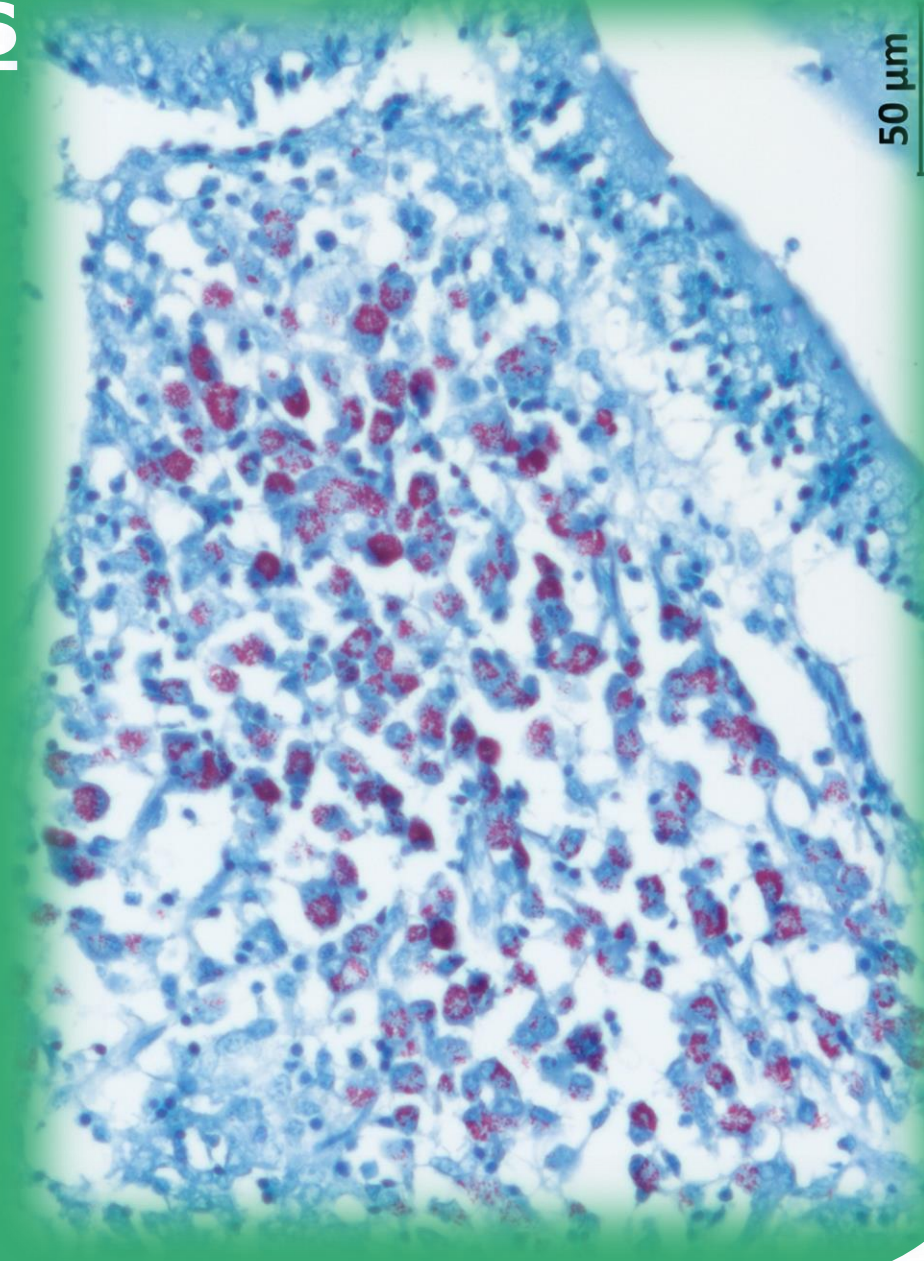
Faeces, ileocecal valve, terminal ileum, mesenteric and ileocecal lymph nodes.



Histopathological analysis

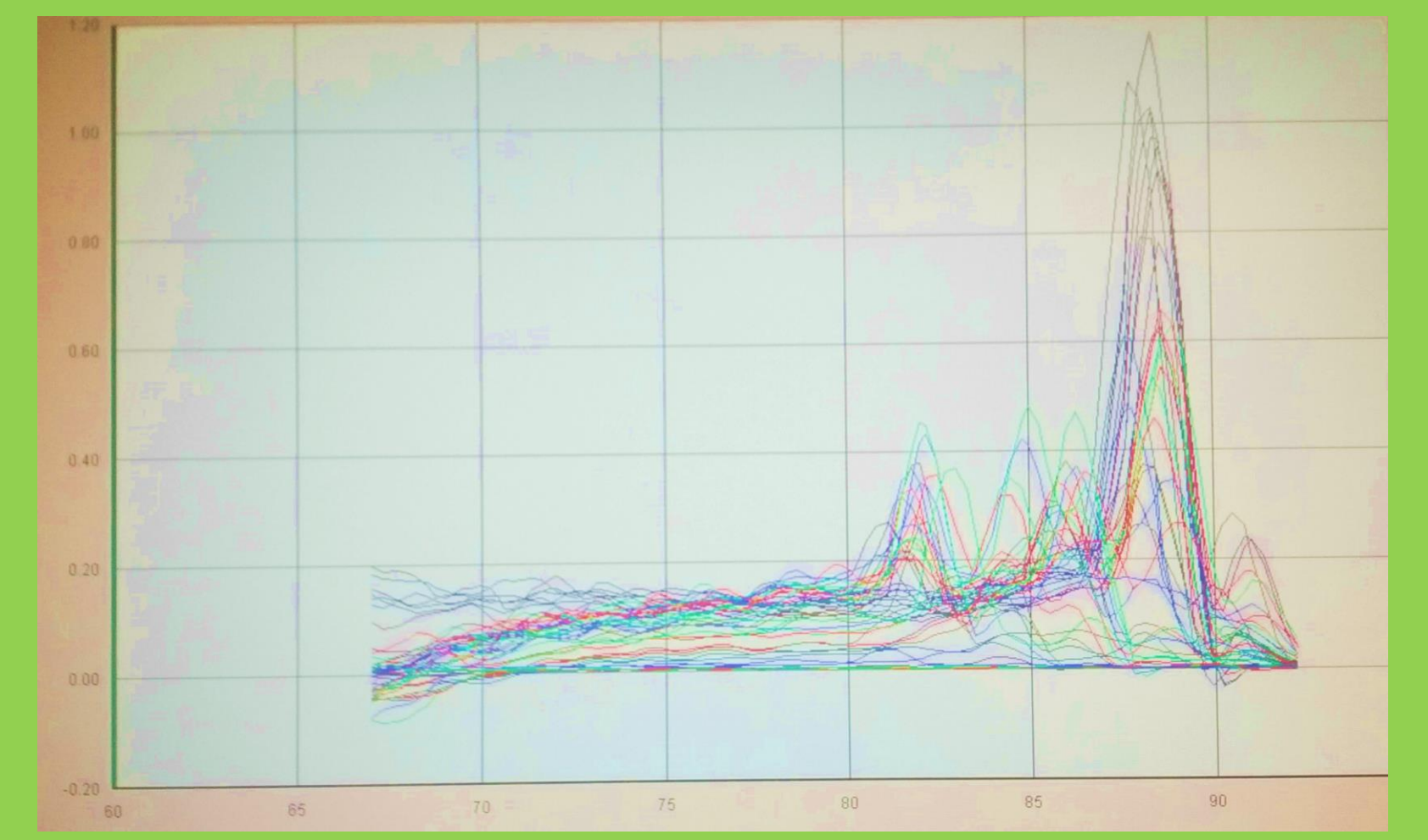
- Tissue fixation: 10% formaldehyde.
- H&E and Ziehl-Neelsen stains.
- Classification of intestinal lesion.

Pérez et al., 1996 and
González J. et al., 2005



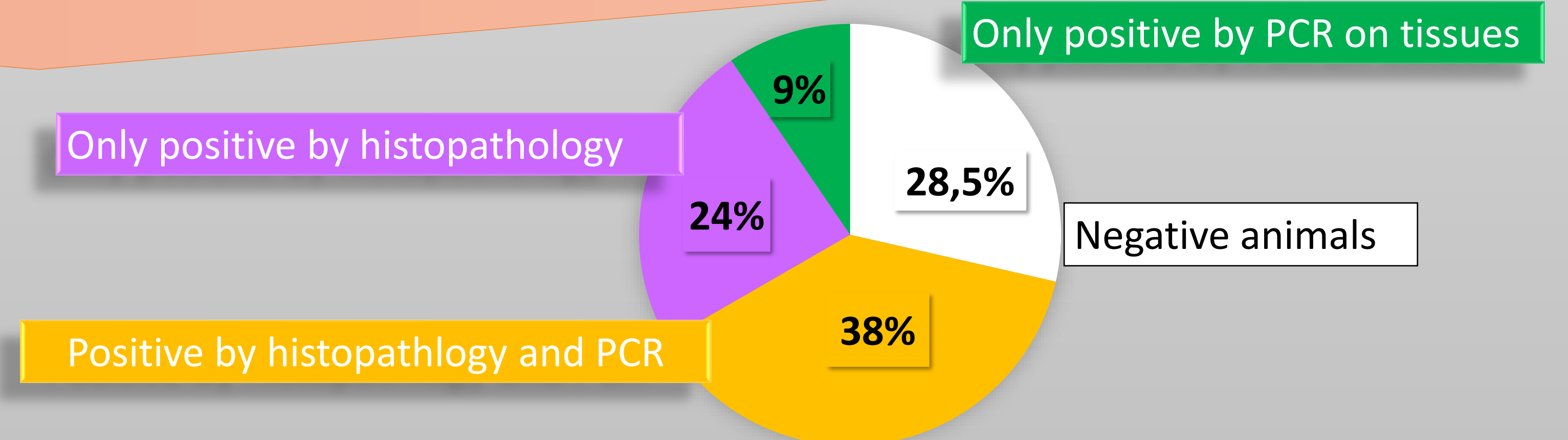
Molecular biology analysis

Real-time PCR (qPCR) for the detection of the Map-specific f57 in faeces and tissue.

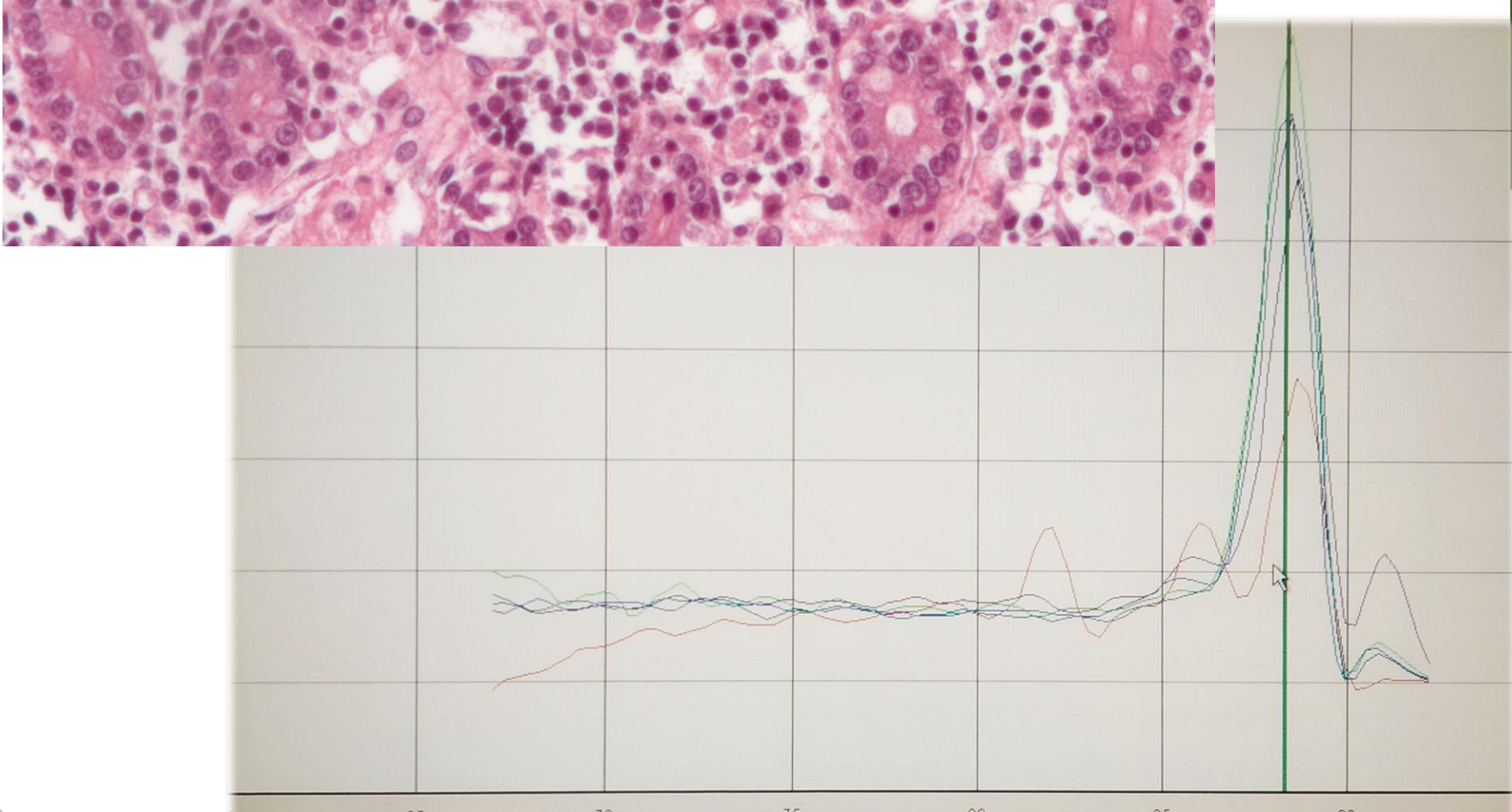
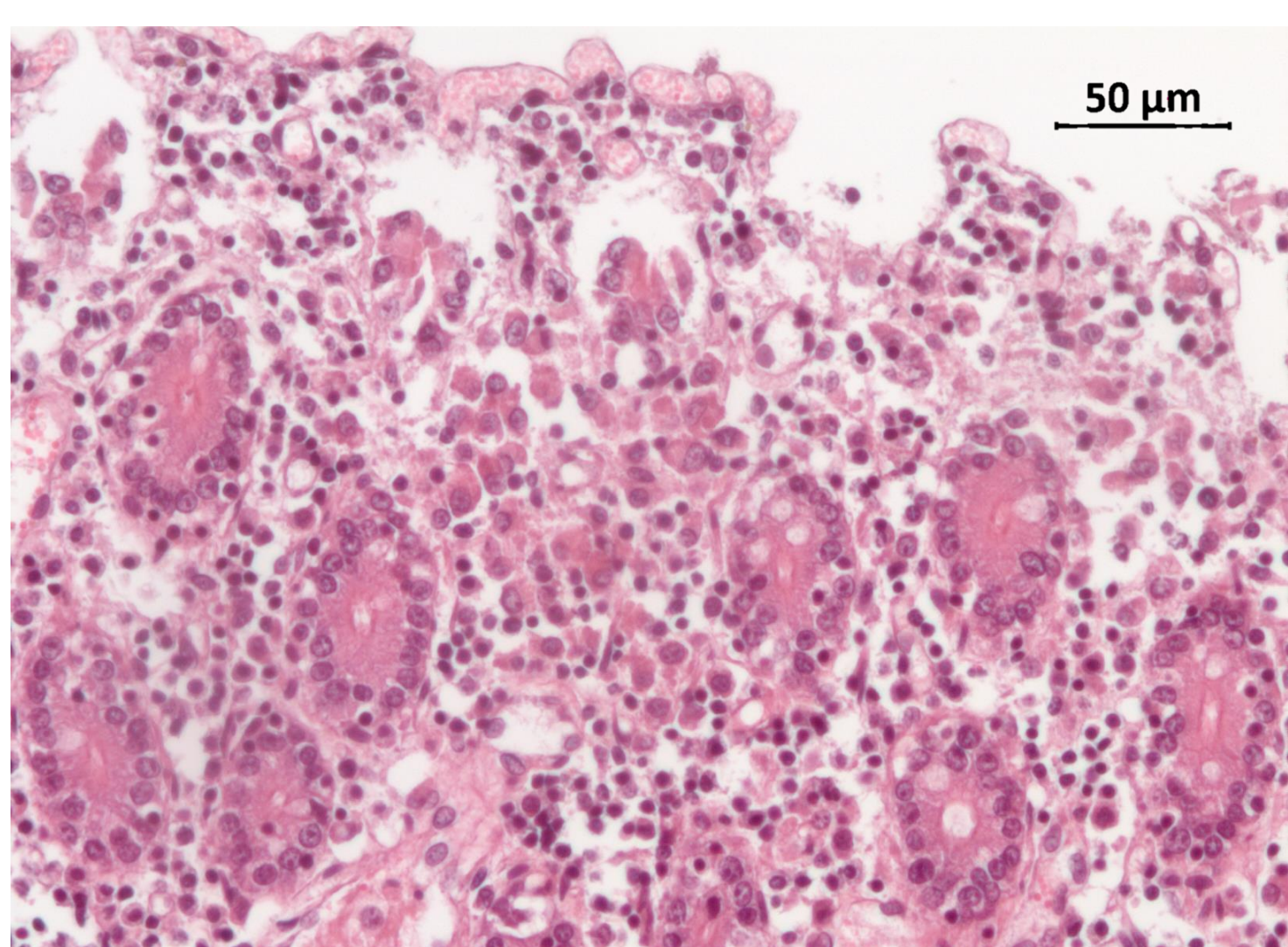


RESULTS AND DISCUSSION

PTB was diagnosed in 71.4% of the goats by any of the tests used (histopathology and qPCR in faeces or tissue).



The 40% of affected animals that were positive in the faecal qPCR mainly exhibited advanced and multibacillary lesions.

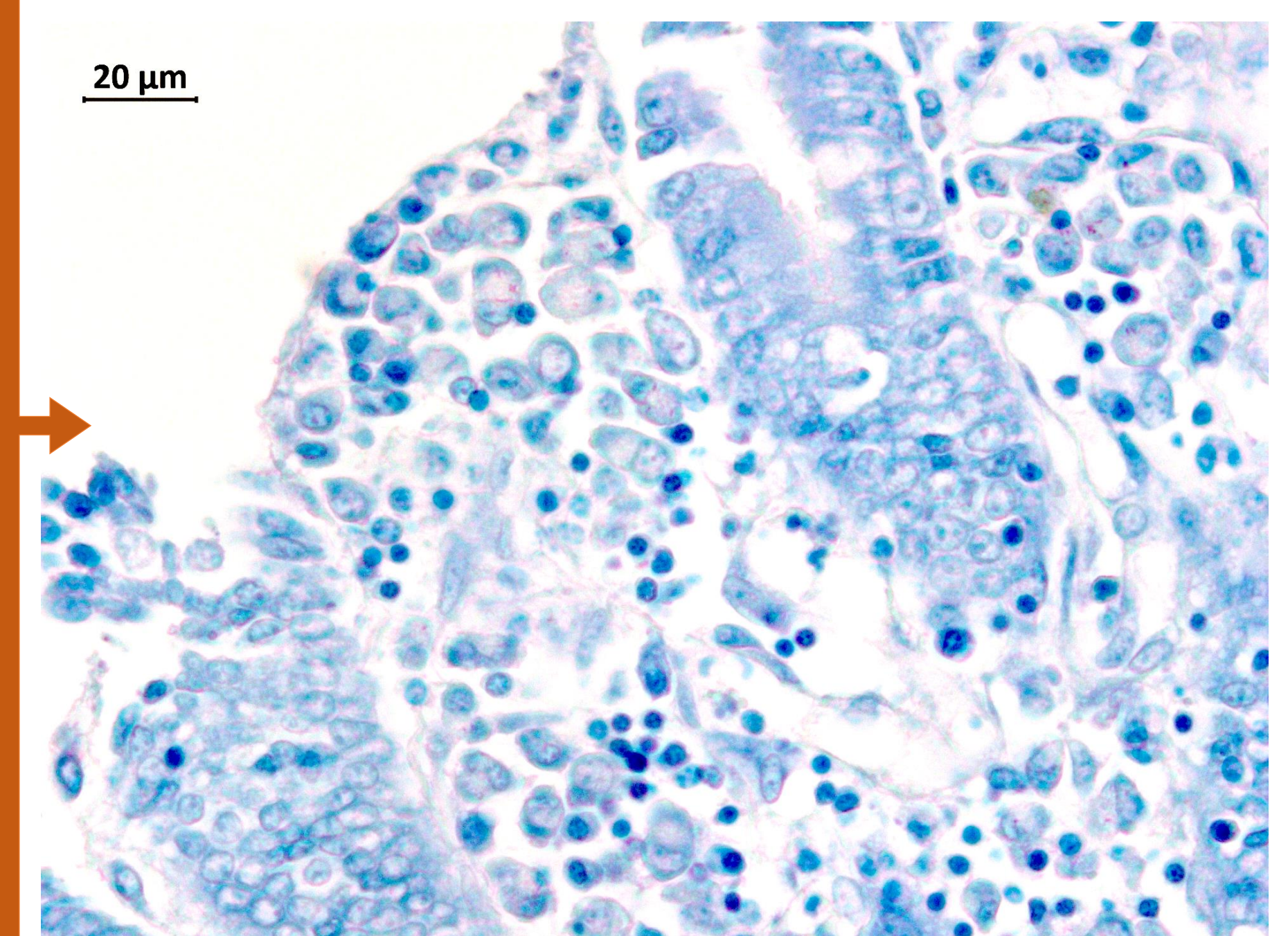


Animals PTB +							
Animal	Positive faecal PCR		Negative faecal PCR				
	Faeces	Lesion	Animal	Faeces			
		H&E	Z-N				
1	Normal	4	1	7	Normal	4	1
2	Fluid	4	1	8	Normal	2	2
3	Doughy	3	2	9	Doughy	2	1
4	Doughy	3	2	10	Normal	1	1
5	Normal	2	1	11	Normal	1	1
6	Doughy	3	2	12	Normal	1	1
				13	Normal	1	1
				14	Normal	0	0
				15	Doughy	0	0

H&E → 0= Absence; 1= Focal; 2= Multifocal;
3= Classic diffuse; 4= Lymphocytic diffuse

Z-N → 0= Absence; 1= Paucibacillary;
2= Multibacillary

However, not all animals with mild and paucibacillary lesions were faecal qPCR positive.



The low sensitivity of faecal-PCR may be related to early stages without bacterial replication yet, animals that overcome the infection or a low bacterial load excretion.

(Whittington & Sergeant E, 2001; Keller et al., 2014; Windsor, 2015)

CONCLUSIONS

PTB affected a high percentage of discarded goats without apparent clinical digestive signs.

The faecal qPCR was highly specific but not very sensitive to detect animals with mild lesions and low bacterial load.