DIAGNOSTIC TEST COMPARISON ON TUBERCULOUS LESIONS FOUND IN SLAUGHTERED CATTLE LYMPH NODES

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HYPOTHESIS AND OBJECTIVES

Hypothesis: In slaughtered bovine lymph nodes with lesions compatible with tuberculosis there is a relation between the histopathological analysis and the positivity to the different diagnostic tests.

Objectives:
- To analyze and classify histologically bTB lesions in lymph nodes from slaughterhouse.
- To correlate the histopathological analysis of these lesions with other diagnostic tests.

INTRODUCTION

Bovine tuberculosis (bTB) is a chronic bacterial infectious disease caused by several species of *Mycobacterium tuberculosis* complex (MTBC). The characteristic tuberculous lesion is the granuloma, found typically in lung and lymph nodes in cattle. For the confirmation of a bTB diagnosis at slaughter, histopathological (HP) analysis, PCR and mycobacterial culture are required. A detailed analysis of histopathological lesions of bTB and the possible correlation with other diagnostic test would provide more information to improve the accuracy of the histopathological diagnosis.
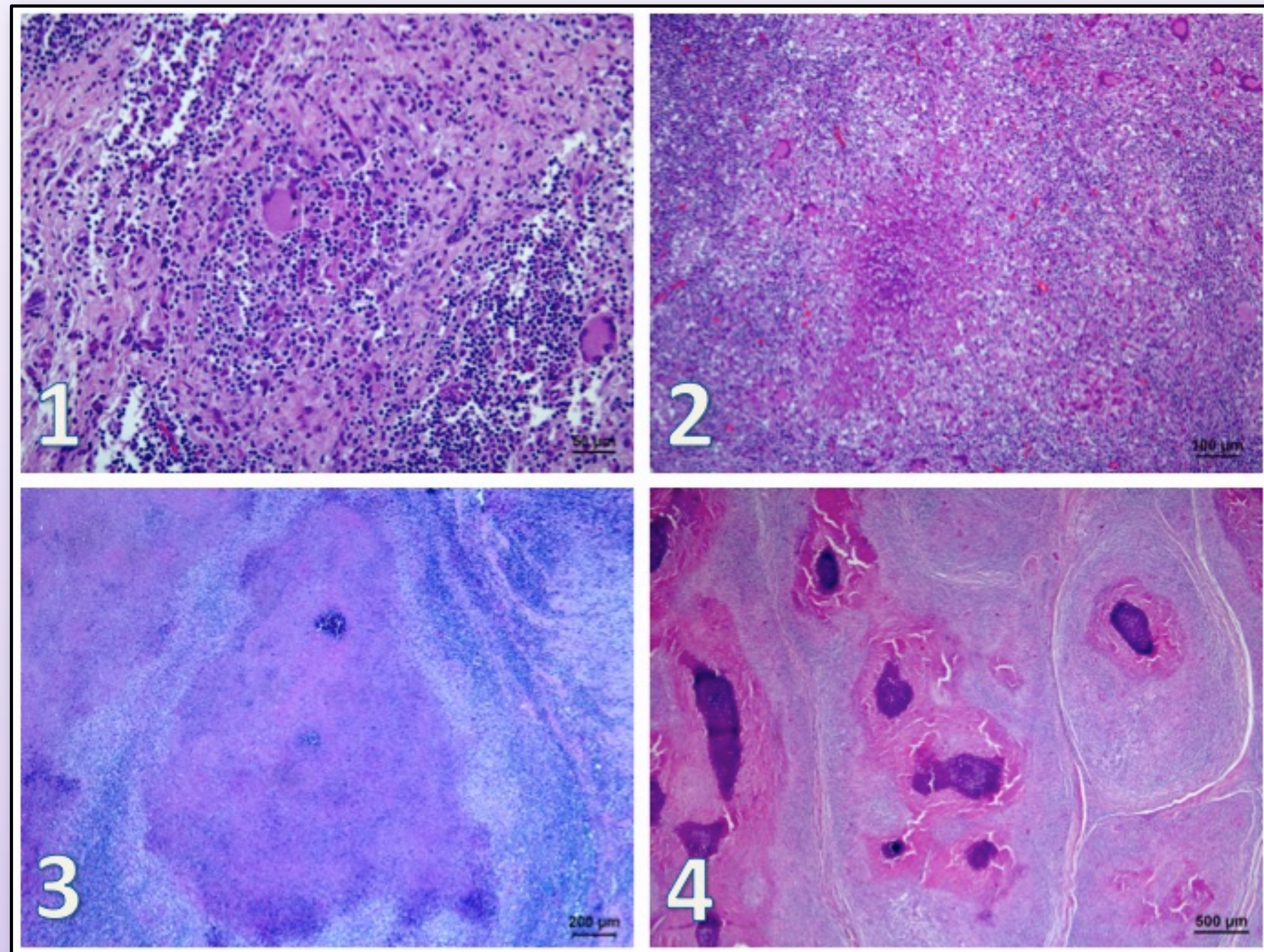
MATERIALS AND METHODS

DATA AND SAMPLES:
- 103 bovine lymph node samples from slaughterhouse confirmed to be tuberculosis, between 2008-2013.
- Results of the diagnostics tests used to confirm bTB: (1) Hematoxylin-Eosin, (2) *Ziehl-Neelsen* (ZN), (3) PCR and (4) Culture (C). DVR-spoligotyping results when available. PCR and culture kindly provided by CReSA-IRTA.

HISTOPATHOLOGICAL ANALYSIS:
- Classification based on grades (I to IV) following the criteria described in previous studies (**Image 1**).
- Semi-quantitative analysis of different microscopic characteristics of bTB lesions (neutrophils, lymphocytes, multinucleated giant cells, macrophages, fibrosis, necrosis, hemorrhage and mineral).

STATISTICAL ANALYSIS:
Data-base:
1. Results from the histopathological examination (**Fig. 1**, **Table 1**).
2. Results from four *post-mortem* tests (HP, ZN, PCR and C) in binary outcome (positive or negative) (**Fig. 2**).
Analysis:
1. Non-parametric exact Fisher test and Chi-square analysis (bivariate analysis), followed by logistic regression (multivariate analysis) to compare:
   - Positivity to the diagnostic tests with different microscopic characteristics (**Fig. 3**).
   - Perceive the relation between the parameter "GRADE" and the other parameters.
2. Sensitivity (SE) and Specificity (SP) for the HP, ZN and PCR, with culture as reference test (**Table 2**).

Image 1 – Histopathological grades (I to IV) of granulomatous lesion in bovine lymph nodes. 1. Grade I (Initial), 2. Grade II (Solid), 3. Grade III (Minimal necrosis), 4. Grade IV (Necrosis and mineralization).

RESULTS AND DISCUSSION

HISTOPATHOLOGICAL ANALYSIS:

Test positives - Grade (ZN+, PCR+, C+; Number of positives vs Granuloma grade)
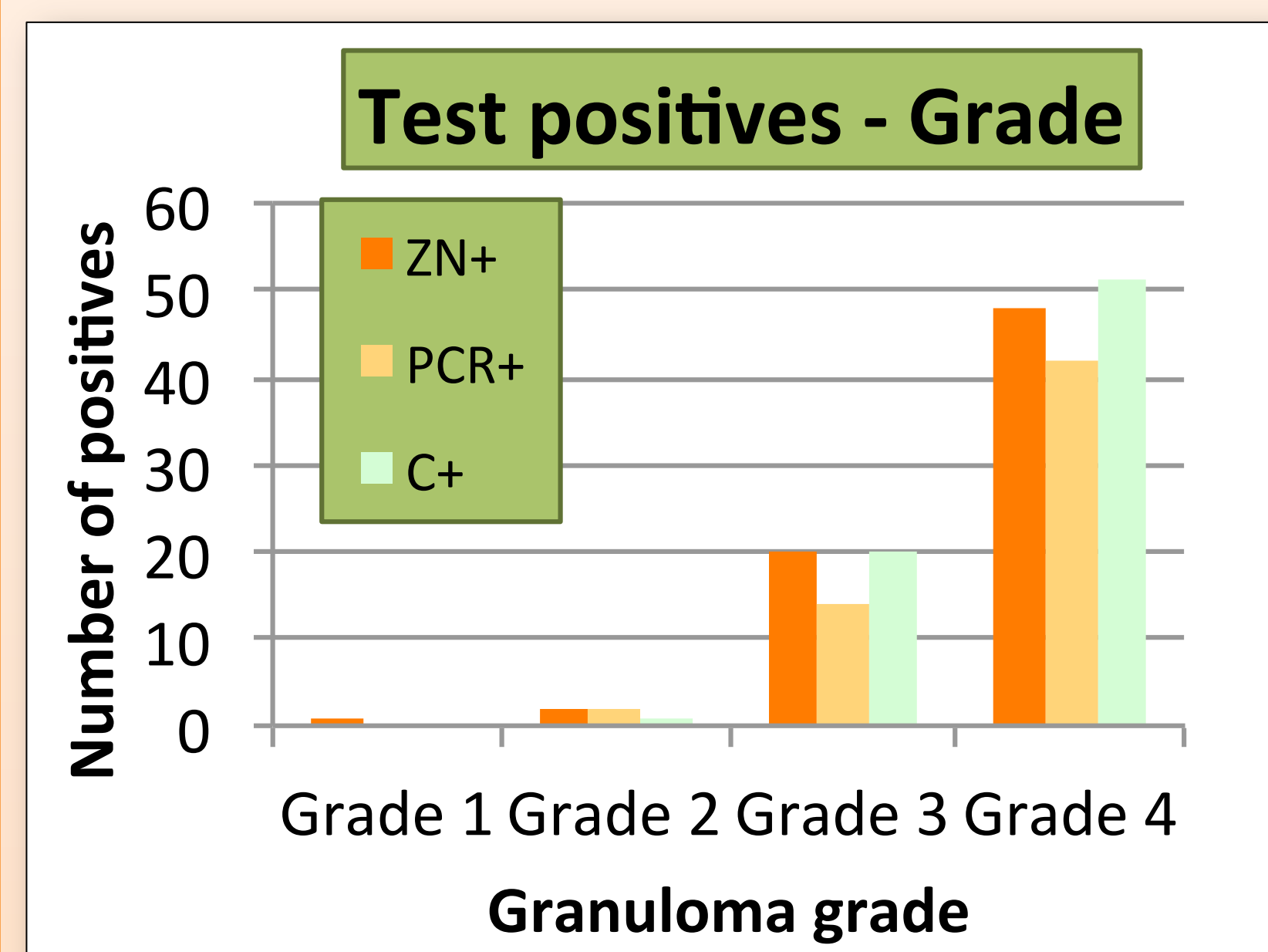
Figure 1 – Positives to diagnostics tests for each histopathological grade. ZN+ = positive to ZN, PCR+ = positive to PCR; C+ = positive to Culture.
Most lymph node granulomas (96 %) presented with advanced lesion development, classified as grade III or IV.
Spoligotyping (of 44/103 samples): *M. bovis* in 36 samples (82 %) and *M. caprae* in 8 samples (18 %).

	PMNN	LIM	MGC	MCF	FIB	NEC	HEM	MIN
Median	1	2	1	2	2	3	0	2
Average	0.65	2.10	1.62	2.20	1.98	2.26	0.18	2.03
SD	0.70	0.53	0.72	0.51	0.85	0.93	0.56	0.95

Table 1 - Histopathological examination. Median, average and Standard Deviation (SD) for each category.

PMNN= neutrophils; LIM= lymphocytes; MGC= multinucleated giant cells; MCF= macrophages; FIB= fibrosis; NEC= necrosis; HEM= hemorrhage; MIN= mineral.

Figure 2 – Frequencies of positive and negative results for ZN (102/103), PCR (90/103) and C (92/103).

Culture: 21,7 / 78,3 (C +, C -)
PCR: 35,6 / 64,4 (PCR +, PCR -)
Ziehl-Neelsen: 30,4 / 69,6 (ZN +, ZN -)
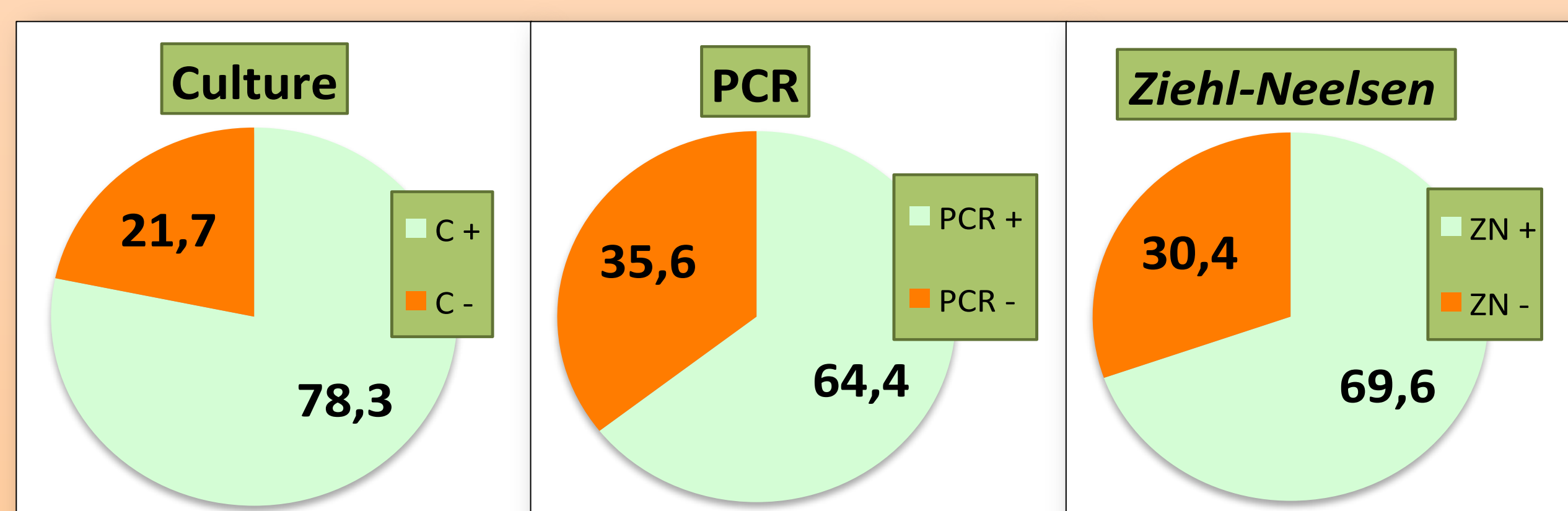
ZN+ = positive to ZN, ZN- = negative to ZN, PCR+ = positive to PCR; PCR- = negative to PCR, C+ = positive to Culture, C- = negative to culture
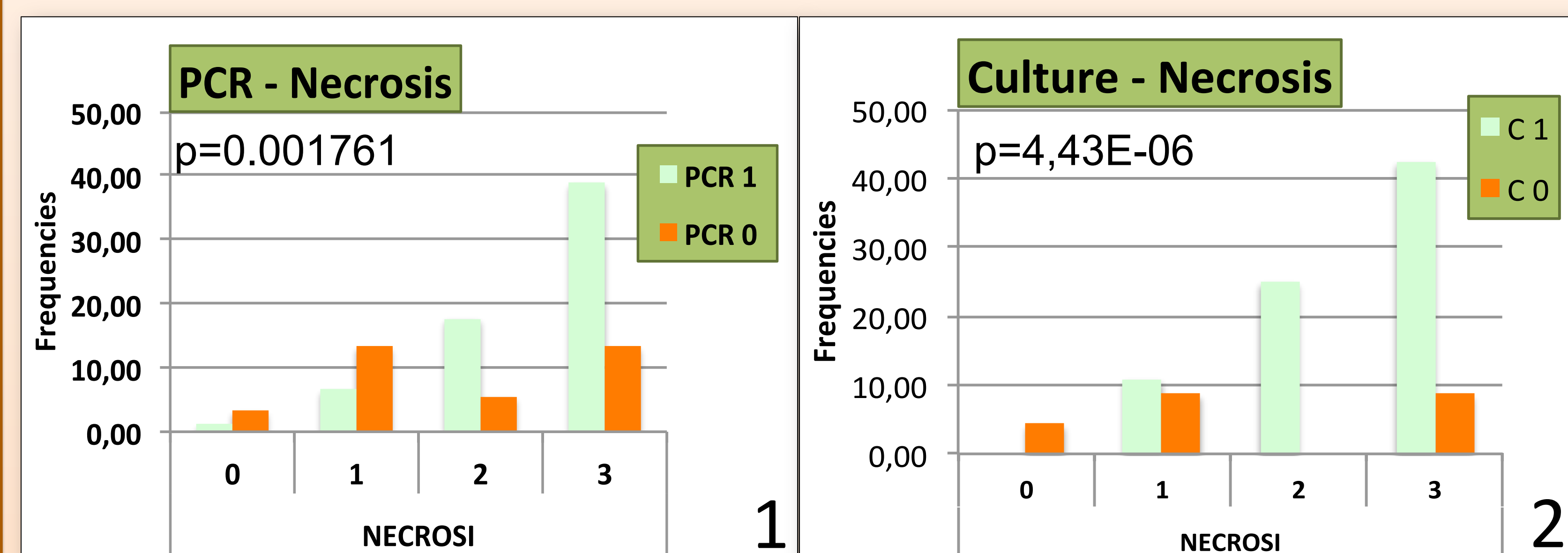
STATISTICAL ANALYSIS:

PCR - Necrosis (p=0.001761; PCR 1, PCR 0; Frequencies vs NECROSI 0-3) — 1
Culture - Necrosis (p=4,43E-06; C 1, C 0; Frequencies vs NECROSI 0-3) — 2

Figure 3 – Bivariate analysis: (1) PCR vs "Necrosis" and (2) Culture vs "Necrosis". Frequencies and p-value. 0-3 parameter score (0=absent, 1=scant, 2=moderate, 3=abundant); PCR (0=Negative, 1=Positive); C= culture (0=Negative, 1=Positive).
"Necrosis" (in score 3; p=0.00354)" and "Mineral" (in scores 1 to 3, p<0.001) were significantly associated with the parameter "GRADE" when performing the multivariate analysis.

	HE	ZN	PCR
Sensitivity	100 %	74.3 %	79.1 %
Specificity	-	55 %	90 %

Table 2 - Sensitivities (SE) and specificities (SP) of the Hematoxylin-eosin (HE), *Ziehl-Neelsen* (ZN) and PCR using mycobacterial culture as a reference test.

CONCLUSIONS

To conclude, detection of bTB lesions at slaughterhouse is basic for the control of the disease and new outbreaks. We observed that most of the analyzed samples corresponded with grades III and IV. We did not find any statistical correlation between the histopathological characteristics of the granulomatous lesions in the lymph node and the positivity to the different diagnostic tests (ZN, PCR and C) used in the *post-mortem* confirmation of a suspected bTB case.