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Melvang, Heidi Mikkelsen; Nielsen, Søren Saxmose; Jungersen, Gregers

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## Association between milk antibody and interferon-gamma responses in cattle from *Mycobacterium avium* subsp. *paratuberculosis* infected herds

Heidi Mikkelsen<sup>1,2</sup>, Søren Saxmose Nielsen<sup>2</sup>, Gregers Jungersen<sup>1</sup>

<sup>1</sup>National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark;

<sup>2</sup>Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark

Contact e-mail: h mz@vet.dtu.dk

### Background

Eradication of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in cattle herds is complicated by lack of accurate diagnostic tests for early diagnosis of infected animals. Available diagnostic methods include detection of MAP by cultivation, cell-mediated immune responses by interferon-gamma (IFN- $\gamma$ ) assays on blood samples or antibodies (Ab) in milk and blood by ELISA.

### Objective

The objective of the present study was to evaluate the association between IFN- $\gamma$  test results in young stock and ELISA status as adult cow among animals with different status in faecal culture (FC).

### Methods

During a three year study period, blood was repeatedly sampled from 15-24 months old heifers in 18 Danish dairy cattle herds infected with MAP, and analysed the following day by a whole blood IFN- $\gamma$  test supplemented with IL-12<sup>(1)</sup>. After calving, milk samples were analysed for MAP Ab by ELISA<sup>(2)</sup> three times per year per animal. For the present analysis, the result of the latest available ELISA test was used. Faecal samples were cultured on Herrold's egg yolk medium<sup>(3)</sup> once per year from adult cattle to describe the MAP shedding status of the cows. Animals were retrospectively grouped by their FC status.

### Interpretation

Animals were considered FC-negative if negative in all samples. The IFN- $\gamma$  test was considered positive if IFN- $\gamma$   $\geq$  1000 pg/ml in PPDj stimulated and IL-12 potentiated blood samples. The ELISA test result was considered positive if OD<sub>Corrected</sub> > 0.3.

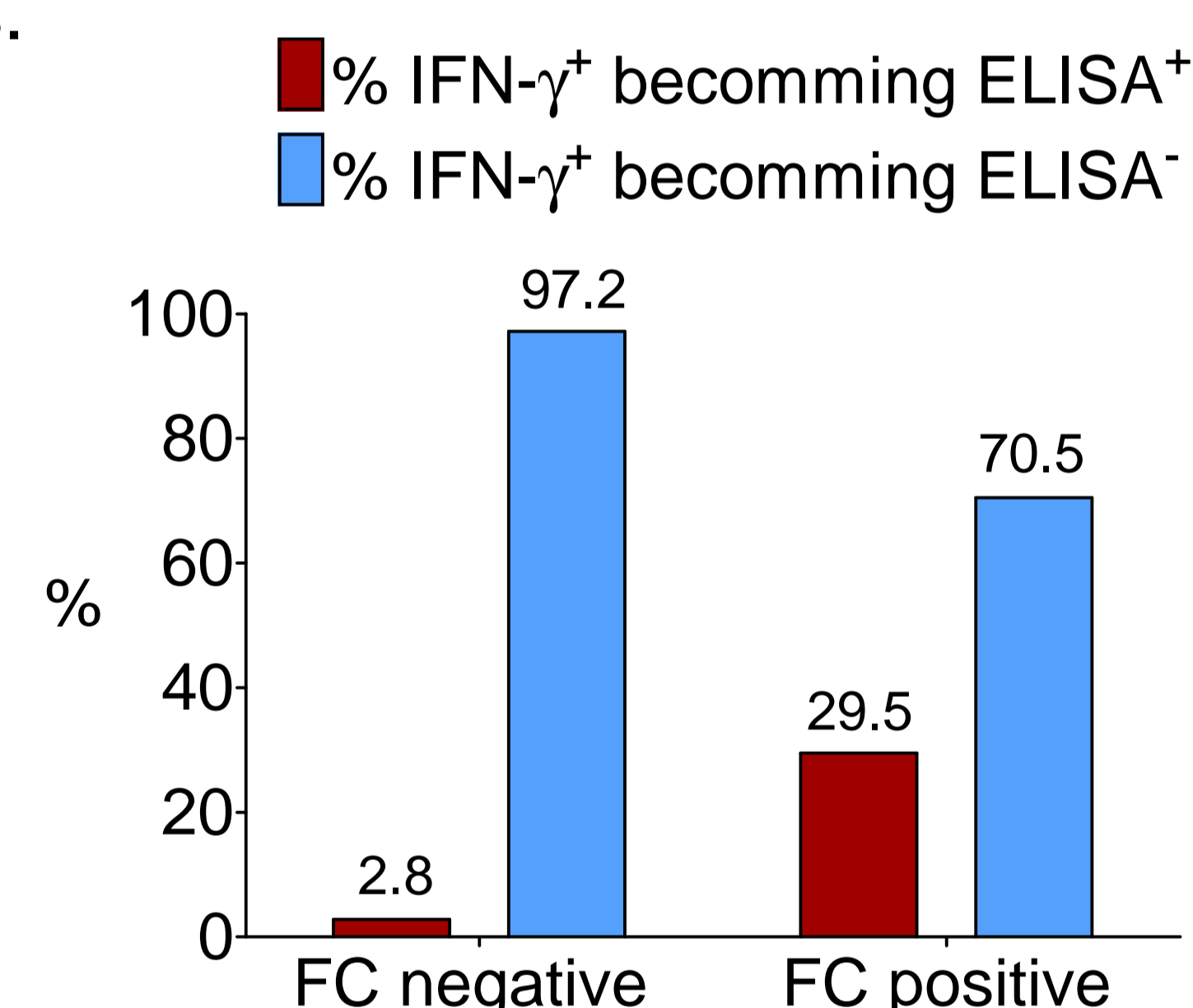


### Results

The distributions of tests-results from ELISA and IFN- $\gamma$  stratified by FC are shown below .

FC positive cows (N= 77)		
	ELISA <sup>+</sup>	ELISA <sup>-</sup>
IFN- $\gamma$ <sup>+</sup>	13	31
IFN- $\gamma$ <sup>-</sup>	11	22
FC negative cows (N= 1180)		
	ELISA <sup>+</sup>	ELISA <sup>-</sup>
IFN- $\gamma$ <sup>+</sup>	17	593
IFN- $\gamma$ <sup>-</sup>	50	520

Among FC negative cows, 2.8% of the heifers, that were previously IFN- $\gamma$  positive, became positive in ELISA, whereas a significantly ( $p < 0.001$ ) higher proportion of 29.5% became ELISA positive among FC-positive cows.



### Discussion and conclusion

The significantly higher proportion of IFN- $\gamma$  positive animals, that tested positive by ELISA among FC positive, correspond to the generally accepted pathogenesis, where animals shedding MAP are more likely to have antibodies. However, among FC-positive, 11 animals (33% of all IFN- $\gamma$  negative) with a positive ELISA were previously IFN- $\gamma$  negative, suggesting that either: a) not all shedding animals are infected as young animals or b) that the IFN- $\gamma$  test is not very sensitive.

This study only included MAP infected herds and hence the specificity of the tests were not evaluated. In addition, the true infection status of cows can at present only be obtained by post mortem histopathology. Moreover, all tests have limitations at certain points during progression of MAP infection. Further evaluation and optimisation of the IFN- $\gamma$  test using new and more specific antigens is necessary for diagnosis in young animals. An association between milk antibody and IFN- $\gamma$  may not be expected until a specific and sensitive IFN- $\gamma$  test has been developed.

<sup>1</sup>Jungersen, G. et al 2005. Conference proceedings, 8ICP

<sup>2</sup>Nielsen, S.S. 2002. Vet. Med. B, 49: 384-387

<sup>3</sup>Nielsen, S.S. et al. 2004. J Appl. Microbiol. 96: 149-153