I prefer:

□ ORAL presentation

☑ POSTER presentation

**Development and evaluation of a triplex droplet digital PCR method for differentiation of M. tuberculosis, M. bovis and BCG**

Yao Qu1, Mengda Liu1\*, Xiangxiang Sun1, Yongxia Liu2, Jianzhu Liu2, Liping Hu3, Zhiqiang Jiang1, Fei Qi1, Wenlong Nan1, Xin Yan1, Mingjun Sun1, Weixing Shao1, Jiaqi Li1, Shufang Sun1, Haobo Zhang1, Xiaoxu Fan1

\*lead presenter

1 Email: liumengda@cahec.cn, China Animal Health and Epidemiology Center, China

2 Shandong Agricultural University, China

3 Shandong Center for Animal Disease Prevention and Control, China

**Abstract:**

**Background/Objective:** Tuberculosis, caused by Mycobacterium tuberculosis complex (MTBC), remains a global health concern in both human and animals. However, the absence of rapid, accurate, and highly sensitive detection methods to differentiate the major pathogens of MTBC, including M. tuberculosis, M. bovis, and BCG, poses a potential challenge.

**Methods:** In this study, we have established a triplex droplet digital polymerase chain reaction (ddPCR) method employing three types of probe fluorophores, with targets M. tuberculosis (targeting CFP-10-ESAT-6 gene of RD1 and Rv0222 genes of RD4), M. bovis (targeting CFP-10-ESAT-6 gene of RD1), and BCG (targeting Rv3871 and Rv3879c genes of ΔRD1), respectively.

**Results:** Based on optimization of annealing temperature, sensitivity and repeatability, this method demonstrates a lower limit of detection (LOD) as 3.08 copies/reaction for M. tuberculosis, 4.47 copies/reaction for M. bovis and 3.59 copies/reaction for BCG, without cross-reaction to Mannheimia haemolytica, Mycoplasma bovis, Haemophilus parasuis, Escherichia coli, Pasteurella multocida, Ochrobactrum anthropi, Salmonella choleraesuis, Brucella melitensis, and Staphylococcus aureus, and showed repeatability with coefficients of variation (CV) lower than 10%. The method exhibits strong milk sample tolerance, the LOD of detecting in spike milk was 5×103 CFU/mL, which sensitivity is ten times higher than the triplex qPCR. 60 clinical DNA samples, including 20 milk, 20 tissue and 20 swab samples, were tested by the triplex ddPCR method and triplex qPCR. The triplex ddPCR presented a higher sensitivity (11.67%, 7/60) than that of the triplex qPCR method (8.33%, 5/60). The positive rates of M. tuberculosis, M. bovis, and BCG were 1.67%, 10% and 0% by triplex ddPCR, and 1.67%, 6.67% and 0% by triplex qPCR, with coincidence rates of 100%, 96.7%, and 100%, respectively.

**Conclusion:** Our data demonstrate that the established triplex ddPCR is a sensitive, specific and rapid method for differentiation and identification of M. tuberculosis, M. bovis, and BCG.

**Keywords:** Molecular diagnosis, multiplex droplet digital PCR, tuberculosis, M. tuberculosis, M. bovis, BCG.