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PROGRAM & ABSTRACTS

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P-03.18 COMPARISON OF COMMERCIAL DNA EXTRACTION AND QPCR SYSTEMS FOR A BETTER SENSITIVITY IN DETECTING THE CAUSATIVE PARATUBERCULOSIS PATHOGEN IN DAIRY COW FECAL SAMPLES

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Mycobacterium avium ssp. paratuberculosis (MAP) is the pathogen inducing rumenitis paratuberculosis (Johne’s disease) worldwide. While formerly of sporadic incidence, the prevalence of infected animals resulting from modern farming has given rise to a new paratuberculosis bovine disease to a global concern. Oral-fecal contamination is the most important mode of transmission of paratuberculosis. Hence, eradicating shedders could prevent MAP propagation. Whereas considered the standard method for MAP diagnosis, fecal culture requires specialised costly media and a long incubation time which sometimes resolve into disappointing bacterial contamination. To facilitate the efforts of control programs we evaluated the performance of direct fecal QPCR assays in terms of sensitivity. Several commercial kits use different strategies for DNA extraction and QPCR systems for capturing the presence of MAP in fecal samples. In this study, our aim is to compare the sensitivity of detection of three commercially available DNA extraction kits broadly used in Canada, namely, A, B, and C, combined with two methods of QPCR detection (T and V). Forty-nine dairy cows from five different herds were diagnosed and sampled by fecal culture and ELISA tests to 6 months interval during two years. Their fecal samples were then tested eight times (8 replicates) with the respective DNA extraction method. While all of the three commercial DNA extraction kits were described as very efficient for the paratuberculosis diagnosis, method B allows a more sensitive detection than the two others. Indeed, 100% of cows declared positive for paratuberculosis by both fecal culture and ELISA assays were identified with method B while only 28% and 41% cows were confirmed with methods A and C, respectively. Interestingly, by using method B, low MAP shedders were detected. Moreover, the QPCR system plays a critical role, with detection system T yielding QPCR reactions with the highest sensitivity. The results presented herein suggest that DNA extraction kit C in combination with QPCR system T allow successful amplification of MAP DNA from fecal samples with the highest sensitivity and specificity. The current study demonstrates the importance of testing different kits for DNA extraction from fecal samples and the impact of a QPCR system to identify MAP shedding animals.

Keywords: Diagnosis, commercial DNA extraction system, fecal sample, quantitative polymerase chain reaction, sensitivity

P-03.19 PERIPHERAL PRODUCTION OF IFN-γ IN GOATS VACCINATED AGAINST PARATUBERCULOSIS IN RESPONSE TO DIFFERENT MYCOBACTERIUM AVIUM SUBSP PARATUBERCULOSIS (MAP) AND M. BOVIS ANTIGENS.

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Animals vaccinated against paratuberculosis could react in immune-based diagnostic methods for other mycobacterial diseases, mainly Mycobacterium bovis infections. The interference with the diagnostic tests used in tuberculosis eradication programs is the main reason barraging the use of paratuberculosis vaccines in cattle in most countries. In this study, different antigens have been evaluated for immune-based diagnostic tests applied to goats vaccinated against paratuberculosis in order to evaluate whether they could be used to avoid cross reactions. Goats vaccinated at 1, 5, 9 months old or when adults, were sampled at 4, 9 and 15 months post-vaccination (mpv) and whole blood was used for IFN-γ release assay. Paired unvaccinated controls were used in each group. For the assays, the following antigens were employed: avian and bovine PPD (CZ Veterinaria), johnin (Neiker), EC and HP M. bovis protein cocktails (Prionics,) and VK055 and VK067 MAP proteins (Vacunek). No significant differences in the IFN-γ release was observed between vaccinated and unvaccinated animals in samples incubated with EC and HP M. bovis proteins or with VK067 MAP antigen. The highest responses were obtained when Johnson and avian PPD were employed, and also with bovine PPD, although always lower than the previous two. Only samples from vaccinated animals incubated with VK055. Map protein gave positive reactions, lower that those observed with johnin or avian PPD. These results suggest that EC or HP M. bovis protein cocktails, already used for tuberculosis vaccination, would be useful for discriminating paratuberculosis vaccinated from M. bovis infected animals.

Keywords: IFN-gamma, Mbovis, vaccination, cross-reaction

P-03.20 EVALUATION OF DIFFERENT IMMUNOLOGICAL METHODS FOR THE DIAGNOSIS OF PARATUBERCULOSIS IN DAIRY COWS AND THEIR RELATIONSHIP WITH THE FECAL SHEDDING DETERMINED BY PCR

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The diagnosis of the infective and the relationship with the fecal shedding of Mycobacterium avium subsp. paratuberculosis (MAP) in dairy cattle, its main reservoir, has been performed by different techniques. In this study, the percentage of ELISA positive animals was significantly higher in adult cattle than in younger animals. In contrast, the number of cattle positive to IFN-γ test was lower among adult cattle. In herd C, only 3 out of 34 cows were positive to ELISA in both milk and serum samples. Map fecal excretion was only identified in the single animal showing positive results to ELISA and IFN-γ test plus clinical signs of paratuberculosis.

According to these results, the number of Map infected animals determined by immunological methods in a herd with paratuberculosis can reach high values. Response to immune-based diagnostic tests is related to the age of the animals and would precede fecal Map excretion.

Keywords: Cattle, diagnosis, immune-based test, fecal excretion

P-03.21 CELL MEDIATED IMMUNE RESPONSE TO L5P IN LONGITUDINAL STUDY OF HEIFERS FROM NATURALLY MYCOBACTERIUM AVIUM SUBSP PARATUBERCULOSIS INFECTED HERD

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Keywords: Lipopentapeptide (L5P), Antigen specific, IGRA, CMI, Predictive diagnosis.