Comparison of milk culture, direct and nested polymerase chain reaction (PCR) with fecal culture based on samples from dairy herds infected with Mycobacterium avium subsp. paratuberculosis

Gao, A; Odumeru, J; Raymond, M; Hendrick, S; Duffield, T; Mutharia, L

Date: 2009

Abstract:

Mycobacterium avium subsp. paratuberculosis (MAP) is the etiologic agent of Johne's disease in cattle and other farm ruminants, and is also a suspected pathogen of Crohn's disease in humans. Development of diagnostic methods for MAP infection has been a challenge over the last few decades. The objective of this study was to investigate the relationship between different methods for detection of MAP in milk and fecal samples. A total of 134 milk samples and 110 feces samples were collected from 146 individual cows in 14 MAP-infected herds in southwestern Ontario. Culture, IS900 polymerase chain reaction (PCR) and nested PCR methods were used for detecting MAP in milk; results were compared with those of fecal culture. A significant relationship was found between milk culture, direct PCR, and nested PCR ($P < 0.05$). The fecal culture results were not related to any of the 3 assay methods used for the milk samples ($P > 0.10$). Although fecal culture showed a higher sensitivity than the milk culture method, the difference was not significant ($P = 0.2473$). The number of MAP colony-forming units (CFU) isolated by culture from fecal samples was, on average, higher than that isolated from milk samples ($P = 0.0083$). There was no significant correlation between the number of CFU cultured from milk and from feces (Pearson correlation coefficient = 0.1957, $N = 63$, $P = 0.1243$). The animals with high numbers of CFU in milk culture may not be detected by fecal culture at all, and vise versa. A significant proportion (29% to 41%) of the positive animals would be missed if only 1 culture method, instead of both milk and feces, were to be used for diagnosis. This suggests that the shedding of MAP in feces and milk is not synchronized. Most of the infected cows were low-level shedders. The proportion of low-level shedders may even be underestimated because MAP is killed during decontamination, thus reducing the chance of detection. Therefore, to identify suspected Johne's-infected animals using the tests in this study, both milk and feces samples should be collected in duplicate to enhance the diagnostic rate. The high MAP kill rate identified in the culture methods during decontamination may be compensated for by using the nested PCR method, which had a higher sensitivity than the IS900 PCR method used.