Molecular Characterization of *Pasteurella multocida* Isolates From Fatal Cases of Bovine Respiratory Disease

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Introduction

Bovine respiratory disease (BRD) is the most costly disease of beef cattle in North America. *Mannheimia haemolytica* is the most common cause of BRD, but recent work suggests that *Pasteurella multocida* is increasing in importance. Because *P. multocida* is a commensal inhabitant of the upper respiratory tract, it is generally considered an opportunistic pathogen. However, studies in swine indicated that there may be a limited number of strains associated with disease, suggesting that some are more virulent than others. We hypothesize that similar strain differences exist in cattle. Polymerization chain reaction (PCR) fingerprinting is effective in discriminating between isolates of *P. multocida*, but it has not been validated in bovine isolates. Therefore, the purpose of this study was to compare the effectiveness of PCR fingerprinting of *P. multocida* isolates from cases of BRD to more traditional approaches, including whole cell protein (WCP) profiles, outer membrane protein (OMP) profiles and serotyping. The information obtained through these techniques was then used to examine isolate diversity to determine if disease was primarily attributable to a limited number of strains.

Materials and Methods

P. multocida isolates were obtained from the lungs of 41 cases of fatal BRD. The PCR assays were conducted using purified DNA, two minisatellites (M13 core and the modified M13 core) and one microsatellite (GTG_5). WCP and OMP were prepared and analyzed by SDS-PAGE. Serotyping was performed using agar gel diffusion. The discrimination index (D, the probability that two unrelated strains randomly selected from the test population would fall into different typing groups) was calculated for each typing method and combinations of each using Simpson's index of diversity. Confidence intervals were calculated for each value of D permitting comparison of the discriminatory power of various typing methods.

Results

All techniques were able to discriminate between the isolates, although the number of unique strain types identified varied by technique. Serotyping resulted in five types. The GTG and M13 core primers each yielded five major types and five sub-types. The modified M13 core primer resulted in eight major types and three subtypes. WCP produced eight major types and eight subtypes and OMP had 10 major types and four sub-types. Combinations of testing techniques resulted in up to 31 types. The characterization techniques produced D values ranging from 0.69 (modified M13 core primer) to 0.85 (M13 core primer). Combining results of all three primers resulted in a D of 0.96 (95% CI 0.94, 0.98), whereas WCP, OMP and serotyping produced a D value of 0.98 (0.95, 1.00). Thus, there was no difference in discriminatory power between PCR and protein characterization approaches. Considering the additional labor involved in collecting, processing and analyzing WCP and OMP, PCR was the preferred technique.

Significance

Work involving *P. multocida* isolates from other species suggests that there may be differences in the strains that cause disease compared to commensal strains. If this is the case in cattle, it could direct work toward virulence factors and guide vaccine improvements. It would also facilitate epidemiologic investigation, looking for carriers of disease strains and potentially aid in identifying animals that are at highest risk for disease. Our work has shown that PCR fingerprinting is an efficient and effective way to study the epidemiology of *P. multocida* isolates from BRD. Moreover, we have established that a potentially diverse population of isolates is associated with fatal BRD. This is consistent with the hypothesis that *P. multocida* is an opportunistic pathogen and a wide range of strains are capable of causing disease in stressed cattle. Future work will characterize the diversity of *P. multocida* isolates from nasal passages of healthy and sick calves. This will permit more definitive conclusions as to whether some strains are more commonly found in diseased than in healthy cattle.