A Rapid Method of Detecting *Pasteurella multocida* in Rabbits from Nasal Swabs Using PCR

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ABSTRACT

Pasteurella multocida is a Gram-negative, non-motile, facultative anaerobic coccobacillus that is an important pathogen in rabbits. Current methods for detecting *P. multocida* infection in rabbits involves culturing nasal swabs on blood agar followed by biochemical analysis for definitive identification. PCR has been used to identify *P. multocida* from colonies grown on plates. A rapid PCR test involving direct PCR of nasal swabs was investigated. Nasopharyngeal cultures were streaked onto TSA with 5% sheep blood and incubated for 24 hours for biochemical identification of *P. multocida*. The swab was then digested in Buccal Amp® and extended using Brilliant® SYBR® Green QPCR Master Mix. We found a strong correlation between PCR positive and culture positive identification for *P. multocida*. PCR positive was indicated by a green florescence at 584 nm. while individual colonies were positive for a biochemical panel specific to *P. multocida*. Based on these data, we conclude that PCR of nasopharyngeal swabs can be used as a rapid, direct method for detecting *P. multocida*.

Key Words: *Pasteurella multocida*, Rabbits, Nasal Swab, PCR

INTRODUCTION

Pasteurella multocida is a Gram-negative, nonmotile, facultative coccobacillus bacterium of considerable historic, economic and scientific importance. In rabbits, *P. multocida* has been implicated in pneumonia, otitis media, conjunctivitis, pyometra, orchitis, abscesses and a generalized septicemia (Flatt & Weisbroth 1990). These diseases are collectively referred to as Pasteurellosis. Pasteurellosis is considered one of the most important diseases in rabbits and is a leading cause of mortality and morbidity. Carrier rates among adult rabbits range from 30-90% and it is generally agreed that *Pasteurella multocida* is universal among conventional rabbitries.

Current methods diagnosing pasteurellosis include nasal cultures, an ELISA (Enzyme-linked immunosorbent assay) test and the use of the Polymerase Chain Reaction (PCR) to detect DNA specific to *P. multocida*. Culture techniques entail taking a nasal or nasopharyngeal swabs and culturing on blood agar. After incubation for 24-48 hours, these cultures are examined for suspected colonies of *P. multocida*. Suspect colonies are re-plated to obtain pure cultures and these cultures are subjected to biochemical tests to identify *P. multocida*. Testing can take 72 or more hours from the receipt of the swab before a definitive diagnosis can be made. In cases where *P. multocida* is present in low numbers, culturing may fail to detect its presence.

In an attempt to overcome the limitations of culturing, an ELISA test (Enzyme-linked immunosorbent assay) was developed. The Elisa tests for the presence of antibodies to *P. multocida* in a blood sample taken from the animal to be tested. Infected animals will produce antibodies to *P. multocida* that can be detected in the blood. While this test greatly reduces the time involved in testing, ELISA tests tend to be expensive and require extensive training in the technique. Another drawback to the ELISA is that it only detects antibodies to *P. multocida* and doesn't tell if the animal is actively infected or merely expressing antibodies to a previous infection. In other words, a rabbit once exposed to *P. multocida* will carry antibodies even when the infection has passed.

The most recent addition to techniques for diagnosing infection by *P. multocida* is the use of the Polymerase Chain Reaction (PCR) to detect the presence of DNA from *P. multocida*. The use of PCR provides a definitive diagnosis. The current use of PCR entails nasal or nasopharyngeal cultures to isolate bacteria to be tested. The suspected bacterial colonies

are subjected to PCR amplification, which will increase the copies of DNA from a pre-determined template. If *P. multocida* is present, the DNA will be amplified to a concentration that can then be detected when the DNA is separated using gel electrophoresis. While being extremely specific, the current use requires the initial culture and the running of gel electrophoresis to visualize the DNA.

In an attempt to speed up the diagnosis using PCR techniques, a direct amplification technique has been tested. This procedure involves the direct amplification of DNA from culture swabs, eliminating the need for pre-culture to obtain colonies for testing. This procedure will allow results to be obtained in a few hours as opposed to days.

METHODS AND MATERIALS

One hundred mature New Zealand White rabbits (*Oryctolagus cunniculus*) were cultured at random from a conventional rabbitry. Cultures were obtained by passing a Calgiswab Type 4 (calcium alginate swab) through the nasal passages to a depth of 4-5 cm (Figure 1). Swabs were immediately streaked onto trypticase soy blood agar containing 5% sheep blood and incubated in a 5% carbon dioxide atmosphere at 37°C for 24-48 hours. *P. multocida* was identified by standard biochemical characteristics. After steaking, the swabs were placed in 50 µl of Buccal Amp® (Epicentre) in 0.5 ml PCR tubes. Swabs were swirled in the Buccal Amp for 10 seconds and the tip of the swab was squeezed against the edge of the tube to extract as much of the supernatant as possible. Template DNA was prepared by digesting at 65°C for 10 min followed by a 3 min cycle at 98°C. PCR assays were run using 12.5 µl aliquots of template DNA in a 25 µl reaction volumes. Commercial mastermix (Brilliant® SYBR® Green QPCR, Strategene, La Jolla, CA) and commercially synthesized primers PM0762 and PM1231 (25 pmol each F/R) were used to amplify the DNA. DNA template amplification conditions designed for the primers and short double-stranded DNA amplification were run after a denaturing at 95°C for 10 min for 40 cycles of elongation of 95°C for 30 sec, annealing at 55-60°C for 1 min, and extending at72°C for 45 sec. Amplified samples were detected under UV light.

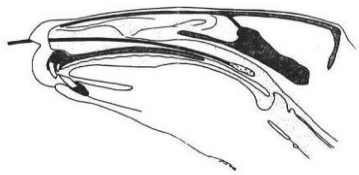

Figure 1. Illustration of the appropriate positioning of a calcium alginate swab for culturing the nasopharynx

RESULTS AND DISCUSSION

Results presented in Table 1 suggest that nasopharyngeal swabs can be used for direct detection of rabbits harboring *P. multocida* in their respiratory tracts. Nasopharyngeal cultures streaked on blood agar have shown to be 38% more effective in detecting carriers of *P. multocida* over conventional nasal cultures while ELISA results proved 20% more effective in detecting infection (Holmes et al. 1987). While ELISA is more effective, it has the disadvantage of being unable to detect active infections vs. previous exposed animals. The PCR of nasopharyngeal swabs was 45% more effective on initial culture at detecting infected animals. Those rabbits PCR positive and culture negative were recultured, and in all cases but one, *P. multocida* was isolated. The need for multiple cultures to find positive animals is eliminated using the PCR technique.

Table 1. Culture and PCR results for the detection of *Pasteurella multocida* in clinically normal rabbits

Position	Negative	Positive
Culture	22	78
PCR	40	60
*Total	40	60

*Total includes rabbits that were positive by culture, PCR or both

REFERENCES

Holmes HT, Matsumoto M, Patton NM, Harris DJ. 1987. A method for culturing the nasopharyngeal area of rabbits. Lab Anim. 21:353-355.