Development of a PCR test for the diagnosis of caseous lymphadenitis in small ruminants

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Introduction

Caseous lymphadenitis (CL), is caused by *Corynebacterium pseudotuberculosis* and leads to issues with fertility, weight gain, and longevity primarily in small ruminants (sheep and goats). CL is responsible for negatively affecting the economy of several countries, including Canada, United States and a reported annual loss of $17 million in Australian wool production (Çetinkayaa, 2002). Moreover, *C. pseudotuberculosis* has caused epidemic levels of infection in thousands of horses during 2002 and 2003 in Kentucky, Wyoming, Utah and Colorado (Foley, 2004).

At the present time, there is no test to diagnose a vaccinated animal before they become symptomatic; unvaccinated animals can be tested using an ELISA test but it is not 100% effective at the animal level (Stanford, 1998, Dercksen, 2000). In addition, the vaccine causes an increase in antibodies against *C. pseudotuberculosis* the ELISA cannot be used in animals that have received vaccine (Stanford, 1998). Typically, CL is diagnosed after the animal is symptomatic, either through biopsy of an affected lymph node or testing pus from an abscess. If the animal becomes symptomatic and the abscess bursts, other animals in the herd are at greater risk of infection.

In an effort to reduce economic impact of an outbreak of CL, this research is intended to develop a reliable DNA-based test to identify the presence of *C. pseudotuberculosis* in blood, saliva/nasal, and/or fecal samples from possibly infected animals. Once the samples are collected, total DNA is isolated, a PCR analysis specific for *C. pseudotuberculosis* is performed. A determining factor in the virulence level of *C. pseudotuberculosis* is the dosage of Phospholipase D (PLD), an exotoxin encoded by the *pld* gene. It is this gene that is detected by PCR, using primers pld-F and pld-R2; this primer set will yield a 203 base pair product (Pacheco, 2007). The drainage material from an enlarged lymph node of an animal infected with *C. pseudotuberculosis* is used as a positive control in this experiment. Once we can determine the specificity of our procedure, we will then attempt to identify *C. pseudotuberculosis* in asymptomatic animals in the same herd using blood, saliva/nasal, and fecal swabs.

Methods

The research methods used were approved by the SUNY Cobleskill institutional animal care and use committee. Lymph node samples of CL infected sheep were collected and stored at 4ºC. Within 24 hours of collection, DNA was extracted directly from the samples using Qiagen DNeasy® Blood and Tissue Kit (#69504, Qiagen, Hilden, Germany). The DNA isolation was conducted according to manufacturer's instructions for tissue based samples. The PCR was performed using a 5 minute denaturation at 94ºC and a hot start for Taq polymerase (#Q82100, Epicenter, Madison, Wisconsin) followed by 30 cycles at 94ºC for 1 minute, annealing at 56ºC for 1 minute and extension at 72ºC for 2 minutes (Çetinkayaa, 2002). The primers used targeted the *pld* gene which encodes PLD. The reverse primer (pld-R2) has a modification on its 3' end (bold in Table 1) that allows for specificity between *C. pseudotuberculosis* and other Corynebacterium (Pacheco, 2006). A 2% TAE agarose gel electrophoresis was conducted under 175 volts for 30 minutes with SybrSafe staining (#S33102, Invitrogen, Carlsbad, CA).

Table 1: Sequence of DNA primers (Pacheco, 2007)

Primer Name	Primer Sequence
PLD-F	5'-ATAAGCGTAAGCAGGGAGCA-3'
PLD-R2	5'-ATCAGCGGTGATTGTCTTCC**AGG**-3'

Figure 1: Infected sheep

This sheep is displaying the cervical swelling (arrow) that is often found in CL affected animals.

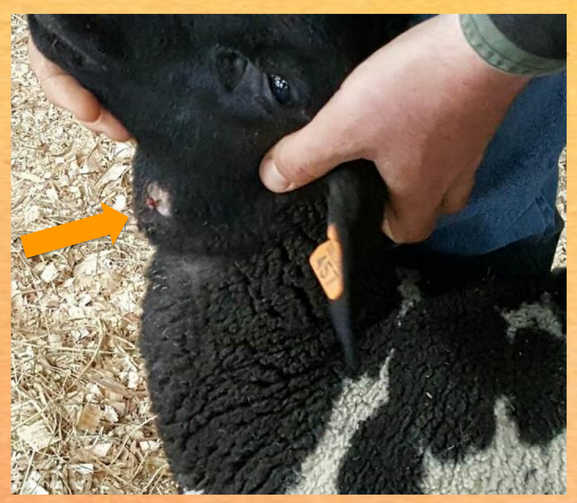

Figure 2: Exudate

Below is an example of the drainage collected from an infected lymph node of a CL affected animal.

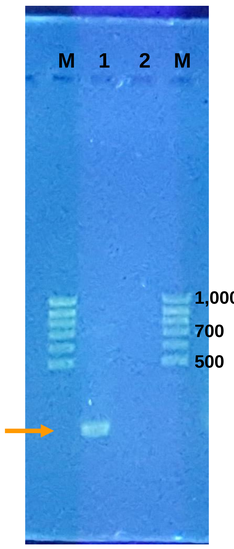

Figure 3: DNA electrophoresis

This is an example of a DNA agarose gel that contains a 1 kb DNA ladder (lane M) for sizing, a PCR sample performed on DNA collected from lymph node drainage (lane 1) and a negative control using water (lane 2). The expected 203 bp fragment is seen in lane 1 (arrow)

Results

Samples were collected from known infected animals (Figure 1) by draining the large cervical abscess and processing the exudate (Figure 2). Whole DNA was isolated from the sample and the *pld* gene specific PCR was performed.

A positive PCR test was observed by a band at 200bp (Figure 3) which is consistent with the expected *pld* gene PCR product (Pacheco, 2007). As a negative control, PCR was performed in the presence of water. The PCR product confirms the presence of *C. pseudotuberculosis* from the provided known positive sample. These results demonstrate that successful DNA isolation and PCR amplification of the *pld* gene of *C. pseudotuberculosis* was accomplished. We are currently examining a sample isolated from a sheep that is thought to have CL in an effort to confirm the diagnosis.

Conclusions

These results confirm the previous work of Pacheco, et al. which demonstrated that these primers could be used on pus or infected lymph node tissue to determine the presence of *C. pseudotuberculosis* (Pacheco, 2007). Our next effort will be to determine if we can detect the PCR product from DNA isolated from blood, saliva/nasal, and fecal swabs of an infected animal. Once we determine that lymph node tissue is not necessary for a positive identification of infection, we can use this method to screen infected and uninfected animals to determine its efficacy. Should this method prove to be effective, animals could then be screened, including those with subclinical disease, so that culling can occur and the disease could be eradicated from the herd.

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