THE DETECTION OF ACHOLEPLASMA SP. IN INDIVIDUAL COW MILK SAMPLES AS "FALSE POSITIVE" GROWTH IN MYCOPLASMA CULTURE

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Introduction

Bovine mastitis caused by Mycoplasma sp. can be an economically significant cause of clinical mastitis and contagious mastitis outbreaks. Mycoplasma culture programs have seen widespread use in commercial dairy udder health strategies and are essential in the prevention and management of this disease. Culturing mycoplasma is usually done on a modified Hayflick media with specialized growth conditions. This culture is frequently performed on bulk tank samples, and milk samples from clinical mastitis cows, fresh cows and new herd additions.

Mycoplasma culture media is a highly selective media, designed to enhance the detection of Mycoplasma sp. The trained analyst can readily distinguish the characteristic morphology of mycoplasma colonies from the few bacterial contaminants that can break through the inhibitor system in mycoplasma culture media. It has been known and previously reported (2) that the environmental saprophyte Acholeplasma Laidlawii is known to occur as a contaminant growth on mycoplasma agar and may have colony characteristics identical to pathogenic Mycoplasma sp. It is therefore recommended that a speciation test service be employed to identify the strain of the isolate. The Udder Health Systems laboratory utilizes the Fluorescent Antibody (FA) test service offered by the Veterinary Medical Teaching and Research Center (VMTRC) in Tulare, California. This secondary speciation test adds significant additional cost and delays to reporting mycoplasma test results. As a practical matter, the FA testing is usually only performed on an initial positive culture from Bulk Tank milk and significantly less frequently from individual cow milk isolates. A total of 223 Bulk Tank Culture isolates have been submitted for FA typing over a 17 year period, from our program, and only three have been identified as Acholeplasma Laidlawii. The operational assumption has been that if mycoplasma growth came from a mastitis cow milk sample then that cow was classified as a mycoplasma cow. Recently, this assumption has been challenged by the potentially costly discovery of “false positive” mycoplasma cultures on two non-mastitis cows that were identified by FA as Acholeplasma Laidlawii. This study was then initiated to attempt to further investigate the nature of this specificity problem in mycoplasma culture.

Materials and Methods

A Digitonin Disk Assay (3) was employed to test 123 isolates from cow milk samples submitted from 14 herds and the results are presented in Table 1. These specimens were submitted to laboratories in Washington and Idaho as part of routine mastitis microbiology testing. The data recorded the colony forming unit (CFU) count estimate based on the inoculation of approximately .01 ml. of milk onto mycoplasma agar. The Digitonin Assay procedure measures the difference in dependence of the isolate on sterols as a nutrient in the agar, to classify them as
either *Mycoplasma sp.* or *Acholeplasma sp.* Several isolates were also submitted to VMTRC for FA confirmation and found to be *M. bovis, M. alkalescens, M. canidense, M. californicum* or *Acholeplasma Laidlawii.*

**Results and Discussion**

Previous research has shown that both low and high CFU counts from mycoplasma quarters can be a valid finding (1). These data show a large number of single CFU and low count positive cultures as actually being affected by *Acholeplasma sp.* contamination, indicating a potentially large false positive problem. When the estimated CFU of culture positives were greater than 100, the assay showed the isolates were almost entirely *Mycoplasma sp.* Cow milk samples from producers with a pattern of *Acholeplasma sp.* contamination, were concurrently cultured on blood agar and were found to be contaminated with a wide variety of other environmental contaminants as well. When a directed effort was made on some of the dairies to improve sample collection technique, *Acholeplasma sp.* findings almost completely ended. *Acholeplasma sp.* was found in all 14 isolates pulled from two bedding samples on one of the affected dairies, with estimated densities of $10^7$ CFU per gram of bedding. The density of this saprophytic growth could be expected to play a role in the likelihood of contamination of cow milk cultures.

The conclusion drawn from these studies is that the presence of *Acholeplasma sp.* can potentially be a significant source of “false positive” reporting if precautions are not taken. The Digitonin Assay in our hands proved to be a useful and robust tool to differentiate true positives from false positives in these samples. When contaminants are found, on farm training in sample collection methods can improve the quality of the submitted samples to mitigate the problem. The influence of *Acholeplasma* growth in bedding needs further evaluation as a source for this contaminant. Laboratories should use contamination evidence from blood agar culture results to advise clients about potential adverse quality effects on their mycoplasma cultures.

Table 1. Colony Forming Units (CFU) of positive mycoplasma culture and classification by Digitonin Assay into *Mycoplasma sp* or *Acholeplasma sp.* on 123 cow milks from 14 herds in Washington and Idaho.

<table>
<thead>
<tr>
<th>CFU Count</th>
<th>Mycoplasma sp.</th>
<th>Acholeplasma sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;100</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>2-99</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>1</td>
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**References**