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Strep species: to differentiate or not to differentiate?

Ynte Schukken, Mary Ellen Charter, Brenda Moslock-Carter, Natalia Belomestnykh and Ruth Zadoks.

More precise culture results led to a more economical antibiotic use on the farms while at the same time maximizing treatment success.

Clinical mastitis in cows is bad enough. Knowing what causes it is certainly helpful in choosing the most appropriate therapy. Hence, we take a milk sample and culture the milk for the presence of bacteria, which often have distinctive characteristics.

We wanted to know if additional tests to differentiate one kind of "strep species" bacteria from another are worth the extra time and cost. A field study we conducted showed that strep differentiation is indeed valuable. More precise culture results led to a more economical antibiotic use on the farms while at the same time maximizing treatment success.

Different bacteria; different treatments

Bacteria in milk often have specific characteristics that make them more or less aggressive and more or less likely to cure. For example, *Escherichia coli* bacteria often cause severe mastitis but may cure relatively easy. On the other hand, Staphylococcus aureus often causes less severe mastitis but is very difficult to cure. These bacteria have been studied extensively and we have relatively good knowledge about the potential treatment programs and the likelihood of success.

Another diagnosis that is often made from a mastitis milk sample is "strep species." Close to 25% of mastitis cases are caused by so-called "strep species." In the case of this bacterial diagnosis we are not so sure about the precise cause of the mastitis case. The diagnosis "strep species" indicates a broad family of bacteria or, in fact, several families of bacteria.

The group is named after the family of streptococci, and each of these streptococcal bacteria may have different characteristics, just as different as *Staphylococcus aureus* and *Escherichia coli.* The main bacterial species that are grouped together in the "strep species" denomination are:

•*Streptococcus dysgalactiae* and S*treptococcus uberis* from the Streptococcus family

• *Lactococcus lactis* from the Lactococcus family

• A number of species from the Enterococcus family (for example. *E. faecalis* and *E. faecium*)

• A large number of other bacteria from these three families and other related families

To get a better idea of these "strep species," a set of further diagnostic tests are necessary to differentiate one species from the next. These additional tests take some extra time and come with additional cost. Thus our question: to differentiate or not to differentiate?

Added value of streptococcus differentiation

The treatment protocols advised for S. dysgalactiae and S. uberis are different. Trials on these two organisms indicate that two or three days of treatment of S. dysgalactiae with beta-lactam antibiotics in registered intramammary tubes such as penicillin, hetacillin or amoxicillin have shown to be successful in 70% to 80% of the cases. Treatment of S. uberis with similar antibiotics would need to be extended to five days to obtain similar results.

Also, antimicrobial resistance patterns for *S. dysgalactiae* typically show very little resistance against common antibiotics, whereas *S. uberis* isolates may show much higher antimicrobial resistance.

Hence, the early diagnosis of *S. dysgalactiae* vs. *S. uberis* provides an important distinction in treatment duration and prognosis for cure.

In the case of *Lactococcus lactis* infection, little is know about the optimal treatment duration and the probability of success. In the case of *Enterococcus* infection, the chance of resistance goes up and the chance of cure goes down.

How does streptococcus differentiation work ?

Conventional culture techniques differentiate bacteria into several groups. These include staphylococci, streptococci and gram-negatives. Usually, subsequent tests further differentiate between several

bacteria within each group. An example would be the coagulase test to distinguish between *Staphylococcus aureus* (coagulase test is positive) or staph species (coagulase test is negative). For the streptococci, no reliable test was used for further differentiation. A recent study that QMPS conducted in collaboration with Keseca Veterinary Clinic and funded by the New York Farm Viability Institute evaluated an additional test to differentiate the streptococci further.

The additional test was a combination of a special *S. dysgalactiae* test (no esculine splitting on blood agar combined with a special test, the so-called Pathodex C) and an additional culture plate, called "Enterococcosel," to identify Enterococcus and Lactococcus. (See figure 1.)

S. uberis and *S. dysgalactiae* do not show growth on this plate because bile salts in the plate stop them from growing. Enterococci and lactococci show growth and dark discoloration of the agar. Some other bacteria may be able to grow on the bile salt plates, but they would not show the dark color, which therefore acts as a second check of the identity of the bacteria.

Figure 1. Enterococcosel plate. The dark zone indicates the growth of bacteria that are not S. dysgalactiae or S. uberis. The most likely bacteria to show such growth would be enterococci or lactococci. The zones marked with S. ub (for S. uberis) and S. dys (for S. dysgalactiae) show no growth.

To evaluate the success of these additional tests, we used a gold standard to provide the correct classification for each identified organism. Using molecular typing techniques, such a gold standard is now readily available. (An example of the molecular typing method using a PCR test is shown in figure 2.) The identified bands for each bacteria provide the final answer for correct streptococcus differentiation.

Figure 2. From left to right the 10 lanes show a molecular ladder, one S. uberis isolate, two S. dysgalactiae isolates, Enterococcus (no product), one S. uberis, three S. dysgalactiae and finally another S. uberis.

Field study

The field study to evaluate the success percentage of this new differentiation protocol was performed in collaboration among four large New York dairy farms, Keseca Veterinary Clinic and QMPS. This part of the study was also funded by the New York Farm Viability Institute. Approximately 200 cases of clinical mastitis with an initial diagnosis was "strep species" were included in the study.

Using the new differentiation protocol, we correctly identified almost 100% of the S. dysgalactiae isolates and over 80% of the S. uberis isolates. Overall, almost 90% of isolates were correctly identified.

For the dairy farmers involved in the study it meant that all *S. dysgalactiae* infections were treated with a relatively short duration of antibiotic therapy while the *S. uberis* and other streptococci (Enterococcus and Lactococcus) were treated with an extended therapy. This resulted in high cure rates for both *S. dysgalactiae* and S. uberis. The study showed the value of strep differentiation to the producers, the veterinarians and the QMPS laboratory staff.

So, differentiate or not differentiate?

The conclusion of this field study supported by the New York Farm Viability Institute was that strep differentiation results in better use of antibiotics, shorter average treatment duration and higher treatment success. We concluded that differentiation of streptococci is advisable. QMPS and Keseca Veterinary Clinic have incorporated the new methodology into routine diagnostics for clinical samples. Further information on implementation of these techniques in your on-farm or in-clinic culture methods may be obtained from any of the QMPS regional laboratories.

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How to reach us...

Dr. Ynte Schukken is director of Quality Milk Production Services in Ithaca, N.Y. Tel: 607-255-8202. Email: yhs2@cornell.edu.

Brenda Moslock Carter, DVM, and Mary Ellen Charter, Veterinary Technician are located at Keseca Veterinary Clinic in Keseca, N.Y.

Natalia Belomestnykh is a laboratory technician at the Ithaca QMPS laboratory.

Ruth Zadoks, DVM, previously head of the QMPS Molecular Laboratory, is now a professor at the Moredun Research Institute in Scotland.

QMPS is a program within the Animal Health Diagnostic Center, a partnership between the New York State Department of Agriculture and Markets and the College of Veterinary Medicine at Cornell.

The QMPS staff of veterinarians, technicians and researchers works with New York dairies to improve milk quality by addressing high somatic cell counts, milking equipment and procedures, and milker training in English and Spanish. QMPS also conducts research and teaching programs.

Reach the four regional QMPS laboratories at:

Central Lab, Ithaca.
877-MILKLAB (877-645-5522)
Eastern Lab, Cobleskill.
877-645-5524
Northern Lab, Canton.
877-645-5523
Western Lab, Geneseo.
877-645-5525
QMPS website:
http://qmps.vet.cornell.edu

Dairy One is an information technology cooperative, providing DHI records services and herd management software to dairies throughout the Northeast and Mid-Atlantic region. A comprehensive laboratory network provides milk quality testing as well as forage, soil, manure and water testing.

Contact Dairy One Cooperative Inc. at 730 Warren Rd., Ithaca, N.Y. 14850. Tel: 800-344-2697. Email: dmr@dairyone.com
Website: www.dairyone.com