STUDY OF CORRELATION BETWEEN DIFFERENT DIAGNOSIS TESTS IN BOVINE MASTITIS

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Summary

In a dairy herd clinical and subclinical mastitis were detected by R-Mastitest and Mast-O-Test, as field tests, and milk samples were collected for bacteriological examination. Between the two field tests used for the detection of clinical and subclinical mastitis and the bacteriological diagnosis there was a positive correlation. There was also a positive correlation between the two field methods.

Bovine mastitis can be diagnosed by Mast-O-Test or R-Mastitest in different phases of evolution, but for implementation of a good treatment, is indicated that the trace and positive reactions in the field tests to be confirmed by bacteriological examination.

In most cases of bovine mastitis, bacteriological, was diagnosed staphylococcal mastitis, most of them were clinical ones.

Key words: field tests, bacteriological examination, bovine mastitis

The economic implication of bovine mastitis, especially the subclinical ones, derives from the high costs, as the increased costs with extra labour for treatment and monitoring, discarded milk from cows with mastitis and from treated cows, loss of milk production, costs of treatment and early culling, control program. A large proportion of mastitis is not detectable only by clinical examination of the udder and milk. Bovine mastitis has also a zoonotic potential by causing food toxicinfections and, in rare cases, a spread of some diseases to humans (tuberculosis, brucellosis). Because of the large number of cases of subclinical mastitis, without a visible modification of the mammary gland and milk, their diagnosis depends on indirect tests (4).

The study aims to investigate the correlation between different diagnosis tests and the causes of clinical and subclinical mastitis in a dairy farm. In the studied unit there is a high percentage of mastitis, and therefore economic losses are important.

Materials and methods

The study was conducted in a dairy farm from Arad County. The growth system is permanent stabulation in winter and free stabulation in summer. In this unit there is a milking hall system.

In this study were collected samples of milk only from 45 cows, without taking into account the cows recently treated, those with physiological status modified and with chronic mastitis. The cows were examined by R-Mastitest and
Mast-O-Test, as field diagnosis tests. All the cows were tested by bacteriological point of view. For the etiological identification and discussion of results were taken into account only samples where were obtained monocultures.

**R-Mastitest** product is used as indirect test for cow’s mastitis diagnosis and it is based on the principle of Californian Mastitis Test - CMT (6).

The reagent used in R-Mastitest consists of sodium laurilsulphate, nonylphenol, polyethylene glycol 4000 and bromcresole purple (as pH indicator). Homogenized milk with an equal amount of reagent results in dissolution and rupture of cell and nuclear membranes. This leads to the release of DNA molecules, which will connect and form a gel. The consistency of gel resulted increases as the number of somatic cells (neutrophiles, macrophages, lymphocytes and epithelial cells) is higher in a mammary quarter.

**How to Perform the Test:** a small sample of milk (approximately 2 ml) from each quarter is collected into a plastic paddle that has 4 shallow cups marked A, B, C and D (corresponding to the four mammary quarters). An equal amount of R-Mastitest reagent is added to the milk. The paddle is rotated to mix the contents. In approximately 10 seconds, read the score while continuing to rotate the paddle. Because the reaction disappears within 20 seconds, the test must be read quickly. The R-Mastitest reagent reacts with the white blood cells and the mixture thickens or gels in proportion to the amount of infection present. To become accurate and consistent, practice this test on cows with a known SCC.

Taking into account the reactive-milk mixture consistency, the **result** can be:

- Negative (-): the mixture remains liquid without precipitation; the mammary quarter is healthy.
- Trace (+): there is a slightly precipitate, the reaction disappears as the paddle is rotated, in about 10 seconds; there is a possible subclinical infection if the precipitate is present in one or up to two mammary quarters.
- Slightly positive (+): there is a distinct precipitate, no gel formation, the reaction can disappear at the paddle rotation over 20 seconds; there is a slightly subclinical infection of the mammary quarter.
- Positive (++): immediately the mixture has a gel consistency, which doesn’t disappear by shaking and levels in the bottom of cup; there is a slightly clinical mastitis.
- High positive (+++): the gel is formed, surface elevates, with a central peak above the mass, this consistency persists at the stopping of paddle rotation; there is a clear clinical mastitis.

Depending on the color of the mixture we have:

- Acid milk (pH=6.3) - yellow;
- Colostrum (pH=6.4-6.5) – yellow - olive;
- Normal milk (pH=6.6-6.7) – gray - violet;
- Mastitic milk (pH=6.8-7.2) – intense purple color.

R-Mastitest results can be directly correlated with the number of somatic...
cells, by the extrapolation and application of the results from CMT, based on the same principle and having similar chemical composition of the reagents. So, we have: negative – 0-200,000 cells/ml, trace – 150,000-500,000 cells/ml, slightly positive – 400,000-1,500,000 cells/ml, positive – 800,000-5,000,000 cells/ml and high positive – over 5,000,000 cells/ml (4).

The Mast-O-Test method is based on the relationship between milk electrical conductivity and its salt and lactose content (2, 3).

In the study it was used the model Mast-O-Test™ 2.0 (8) composed of a measuring cup with terminals (equipped with two graphite electrodes and a temperature sensor), electronic unit (which operates with 3.5V lithium batteries – sufficient for 50,000 readings), switch and handle. The electronic package uses programmed integrated circuits and four sets of LED for ease of interpretation of quarter comparison. The instrument is sealed and waterproof.

Operating mode: after removal of the first streams of milk, put about 5 ml of milk into the cup, switch on and the value measured is displayed on a scale of 00 to 99 units. After 4 seconds, the value for one mammary quarter on the display is stabilized and the appropriate LED (red/orange/green) corresponding to the quarter comes on and remains so for 4 seconds. The above operation is repeated for each mammary quarter. Finally, by pressing the switch again, all four LED display the results of each quarter and, on the digital display, one or more bars corresponding each to a specific quarter will come on if the quarter difference is 15% higher than the lowest one. Quarter samples with readings showing conductivity increase higher than 15% than the lowest reading are an indication of development of subclinical or clinical mastitis.

The color of LED corresponds to the measured values and indicates the severity of subclinical infections, based on the high level of salt in the milk. So, readings below 54 units (green light) indicate a healthy mammary quarter (an average of 150,000 somatic cells/ml milk), readings between 54 and 70 units (orange light) indicate a progressively increasing incidence of subclinical infection (an average of 400,000 somatic cells/ml milk) and readings above 70 units (red light) indicate a rapid increase in the severity of subclinical infections (1 million to many millions of somatic cells/ml milk).

The bacteriological examination of milk samples was realized at Discipline of Infectious Diseases of the Faculty of Veterinary Medicine Timisoara. It included smears from lactate secretion stained by Gram method and culturing the milk samples on broth and blood agar 10%, incubated at 37°C, 24h, in aerobic conditions. Bacteriological typisation of the pathogens was realized on the base of morphological, cultural and biochemical characteristics, according to the methodology described in the scientific literature (1, 4, 5).

Bacteria identified as presumptively belonging to the genera Staphylococcus and Streptococcus in bacterioscopic and bacteriologic examinations were subsequently identified using API-Staph (for genus Staphylococcus) and API 20 Strep (for genus Streptococcus). The API system
enables the classification of strains in species based on biochemical features. For the etiological identification were studied only monocultures.

Results and discussions

There were collected 45 milk samples, from which only 9 were monocultures, 18 were bacteriological sterile and 18 indicated polymicrobial mastitis (in bacteriological exam there were obtained two kind of colonies or more). For the discussion of result and etiological identification we did not take into account that 18 polymicrobial cultures.

From those 9 cows with monoculture (Table 1):
- 5 presented clinical mastitis (milk modification: 3 serous secretion, 1 serous secretion with clots, 1 seroemorrhagic secretion with flakes);
- 4 presented subclinical mastitis (the aspect of milk was apparently normal).

From the 5 cases with clinical mastitis:
- all of them were positive (2 slightly positive, 1 positive, 2 high positive) in R-Mastitest;

From the 4 cases with subclinical mastitis:
- 3 were positive (2 slightly positive, 1 positive) and 1 was trace in R-Mastitest;
- 3 were positive and 1 negative in Mast-O-Test.

Table 1

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Age (years)</th>
<th>Mammary quarter sampled</th>
<th>Mastitis type</th>
<th>Bacteriological examination</th>
<th>Mast-O-Test</th>
<th>R-Mastitest</th>
</tr>
</thead>
<tbody>
<tr>
<td>4203</td>
<td>8</td>
<td>RF</td>
<td>Clinical</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3778</td>
<td>4</td>
<td>RF</td>
<td>Subclinical</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3857</td>
<td>3</td>
<td>RH</td>
<td>Subclinical</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>4298</td>
<td>8</td>
<td>LF</td>
<td>Subclinical</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4210</td>
<td>5</td>
<td>LF</td>
<td>Clinical</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4244</td>
<td>10</td>
<td>LF</td>
<td>Clinical</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>4129</td>
<td>7</td>
<td>RF</td>
<td>Clinical</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>3809</td>
<td>6</td>
<td>LF</td>
<td>Clinical</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>3457</td>
<td>4</td>
<td>LF</td>
<td>Subclinical</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

RF- right front, LF- left front, RH- right hind

From the 9 mammary quarters diagnosed with clinical and subclinical mastitis by bacteriological examination, in R-Mastitest 8 were positive and 1 trace and in Mast-O-Test 8 were positive and 1 negative.

Six of the isolates were presumptively classified in the genus *Staphylococcus*, two in the genus *Streptococcus* and one as *Candida spp*. The API system used in the case of strains presumptively classified in the genera *Staphylococcus* (API-Staph) and *Streptococcus* (API 20 Strep) allowed their
classification in species based on biochemical features (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Serial no.</th>
<th>Mammary quarter sampled</th>
<th>Mastitis type</th>
<th>Milk aspect</th>
<th>Etiological agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>4203</td>
<td>RF</td>
<td>Clinical</td>
<td>serous</td>
<td><em>Staphylococcus aureus</em> subsp. <em>aureus</em></td>
</tr>
<tr>
<td>3778</td>
<td>RF</td>
<td>Subclinical</td>
<td>apparently normal</td>
<td><em>Staphylococcus epidermidis</em></td>
</tr>
<tr>
<td>3857</td>
<td>RH</td>
<td>Subclinical</td>
<td>apparently normal</td>
<td><em>Streptococcus dysgalactiae</em> subsp. <em>dysgalactiae</em></td>
</tr>
<tr>
<td>4298</td>
<td>LF</td>
<td>Subclinical</td>
<td>apparently normal</td>
<td><em>Streptococcus dysgalactiae</em></td>
</tr>
<tr>
<td>4210</td>
<td>LF</td>
<td>Clinical</td>
<td>serous</td>
<td><em>Candida spp.</em></td>
</tr>
<tr>
<td>4244</td>
<td>LF</td>
<td>Clinical</td>
<td>serous with clots</td>
<td><em>Staphylococcus aureus</em> subsp. <em>aureus</em></td>
</tr>
<tr>
<td>4129</td>
<td>RF</td>
<td>Clinical</td>
<td>serous</td>
<td><em>Staphylococcus aureus</em> subsp. <em>aureus</em></td>
</tr>
<tr>
<td>3809</td>
<td>LF</td>
<td>Clinical</td>
<td>serohemorrhagic with flakes</td>
<td><em>Staphylococcus aureus</em> subsp. <em>aureus</em></td>
</tr>
<tr>
<td>3457</td>
<td>LF</td>
<td>Subclinical</td>
<td>apparently normal</td>
<td><em>Staphylococcus aureus</em> subsp. <em>aureus</em></td>
</tr>
</tbody>
</table>

In cases of staphylococcal mastitis were isolated 5 strains of *S. aureus* subsp. *aureus* (coagulase positive) and 1 strain of *S. epidermidis* (coagulase negative). The implication of *S. epidermidis* (microbiota of normal skin) in mastitis is noticed also in the literature (7). Even if *S. epidermidis* is frequently isolated together with other pathogenic bacteria in the mammary glands, with clinical infections, its presence alone in mammary glands still produce an inflammatory response with somatic cells increasing, without clinical expression.

Conclusions

R-Mastitest presented 100% sensitivity for clinical and subclinical mastitis, because all of the reactions have indicated the possibility of intramammary infections, including the trace reaction which was observed only in one mammary quarter.

Mast-O-Test had a sensitivity of 88.88%, only one sample being negative in this field test, by taking into account both clinical and subclinical mastitis.

The Mast-O-Test sensitivity was higher in the cases of clinical mastitis (100% - all of the 5 samples were positive) and smaller in the cases of subclinical mastitis (75% - only 3 samples were positive).

Bovine mastitis can be diagnosed by Mast-O-Test or R-Mastitest in different phases of evolution, but for implementation of a good treatment, is provided that positive and trace results to be bacteriologically confirmed, including the antibiogram.

These field tests can be used with good results for mastitis survey in dairy herds, being simple and quick to apply.
The most frequent pathogen isolated from bovine mastitis was *Staphylococcus aureus subsp. aureus*. Most bovine mastitis caused by this etiological agent were clinical expressed, only one being subclinical.

Between the bacteriological examination and the two field methods for detecting subclinical and clinical mastitis there is a positive correlation, meaning that only 1 result was different, being trace in R-Mastitest and negative in Mast-O-Test.

There was also a positive correlation between the two field tests, because 8 samples were positive in both R-Mastitest and Mast-O-Test. Only one sample had a different result.

References