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Original research paper

TREATMENT OF SUBCLINICAL STAPHYLOCOCCAL MASTITIS

Ožbalt Podpečan [1*], Andrej Pengov [2], Uroš Hrastnik [3]

Addresses of authors: [1] Savinjska Veterinary Station, Celjska c. 3/a 3310 Žalec, Slovenia; [2] Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia; [3] Veterinary Surgery, Farma d.o.o., Podlog 1, 3311 Šempeter, Slovenia

*Corresponding author. E-mail: svp.zalec@siol.net

Summary: The purpose of this study was to evaluate the efficacy of a treatment of subclinical mastitis in dairy cows, caused by the *Staphylococcus aureus* bacteria strain. In both Europe and the USA *S. aureus* is the most frequently isolated pathogen present in dairy cows suffering from subclinical mastitis. Studies have shown that amoxycillin, in its own right, is not sufficiently effective in combating S. *aureus*, however, when used in conjunction with clavulanic acid its efficacy improves significantly. The infected animals were treated with Synulox®, which contains amoxycillin and clavulanic acid. In accordance with the manufacturer's instructions, the treatment was applied intramuscularly and intramammarily. In total, 61 mammary glands of 37 cows were treated. On average, the bacteriological efficacy of the treatment was 51.3 %. In animals with only one infected mammary gland the efficacy was 69.9 %. Considering that such animals represented 56.7 % of all the animals included in the study, we suggest that treating animals with only one or two infected mammary glands with Synulox® is sensible and economically justifiable.

Key words: veterinary medicine; mastitis-treatment; *Staphylococcus aureus;* amoxycillin; clavulanic acid

Introduction

The subclinical form of mastitis in dairy cows represents a significant problem in contemporary milk production, as it is associated with lower productivity and an increase in the somatic-cell count (SCC) in milk. Milk with an elevated SCC is of a lower quality due to an alteration in the quantity of the single ingredients (fat, protein, lactose, and minerals). The efficacy of subclinical-udder-infection treatments mainly depends on the species of the infectious agent and the duration of the infection. Other factors, such as the age of the animal, the preparation of the udder before milking and nutrition, also play an important role in the outcome of a mastitis treatment (2, 4).

While it is true that the percentage of animals suffering from subclinical mastitis in Slovenia is decreasing (31.3 % in 1990 compared to 21.9 % in 1997), the efficacy of treatments of such infections is also diminishing. This is as a result of the increasing resistance of microorganisms to antibiotics, and to the weaker immune systems of the animals due to increased milk production and management failures (1).

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Staphylococcus aureus (S. aureus) is, besides coagulase–negative *Staphylococcus* species, the most frequently isolated pathogen present in dairy cows suffering from subclinical mastitis in both the USA and Europe (2). On average, such infections in Slovenia account for half of all cases, however, this varies significantly within individual herds (1). In this study the *S. aureus* bacteria was more prevalent in the mammary glands of the older animals.

The outcome of a treatment of a mammary gland infection caused by *S. aureus* is very uncertain and is influenced by the age of the animal, the clinical type of infection (acute vs. chronic), the sensitivity of the infectious agent to certain antibiotics and the duration of the treatment.

In cases of chronic infection, the ability of the antibiotics to penetrate the affected tissue is poor. The fact that *S. aureus* can also be present intracellularly and in micro-abscesses represents an additional problem. In those areas it is particularly hard to achieve the appropriate concentrations of the antibiotic (3, 4).

The sensitivity of *S. aureus* to various antibiotics has diminished over time (1, 5), while the share of β-lactamase-positive strains has increased. The percentages vary significantly from country to country and ranges from 4 % in Norway to 76 % in Ireland. Very early in the devel-

opment of antibiotics an enzyme, which destroys penicillin, was described. The enzyme was termed "penicillinase" (now referred to as β-lactamase) and was found to be produced by a wide variety of bacteria. This still remains the most important method of bacterial defence to the β-lactam antibiotics (penicillins, cephalosporins etc.) (5, 6, 7, 8).

The duration of a therapy can also significantly influence the efficacy of the treatment. Acceptable results are achieved through a combination of intramuscular and intramammary applications of appropriate antibiotics over a 3 to 5 day period (9, 10).

According to other authors, the rate of success of bacteriological cures for clinical and subclinical mastitis caused by *S. aureus*, ranges from 15 % to 70 % (2, 7, 9, 10), which indicates the level of difficulty and complexity involved in the approach to treatment.

Amoxycillin in combination with clavulanic acid, which is a β-lactamase inhibitor, is one of the antibiotics that are being used with increasing regularity in the treatment of subclinical bovine mastitis. This combination was first successfully applied in human medicine in the treatment of infections caused by β-lactamase-positive strains of *S. aureus.*

Authors agree that amoxycillin in its own right is not sufficiently effective in combating *S. aureus* (7, 10, 12), however, when used in combination with clavulanic acid the efficacy of a treatment improves significantly.

Considering all the aforementioned facts we decided to test the efficacy of the amoxycillin-clavulanic acid combination in the treatment of subclinical mastitis in dairy cows caused by *S. aureus.*

Material and methods

Selection of Animals

The study included 37 dairy cows from 11 different herds, of different ages (Graph 1) and breeds, each with an increased SCC in their milk. In all cases, a microbiological test of the milk sample revealed the presence of *S. aureus.* The average SCC in milk from the infected udder quarters of the selected animals was $1428x10^3$/ml before treatment, and ranged from $210x10^3$/ml to $4057x10^3$/ml. Twenty-one animals had one infected mammary gland, 9 had two, 6 had three and in one case all four mammary glands were infected. In total, 61 mammary glands of 37 cows were included in the study.

Treatment

Synulox®, which is manufactured by Pfizer Animal Health, was used to treat the infection as follows:

- an injector with an intramammary solution containing 50 mg of clavulanic acid in the form of potassium clavulanate, 200 mg of amoxycillin in the form of Amoxycillin-Trihydrate and 10 mg of prednisolone.
- a solution for an intramammary application where 1 ml contains 35 mg of clavulanic acid in the form of potassium clavulanate and 140 mg of amoxycillin in the form of Amoxycillin-Trihydrate.

All the animals included in the study were treated in accordance with the following predetermined protocol:

- an application of the Synulox® injector into the affected udder quarter every 12 hours at 6 consecutive milkings;
- a parenteral application of the Synulox® solution, in the amount of 8.75 mg/kg, on the first and second days of the treatment with an interval of 24 hours between the doses.

Eleven days after the final application of the drug, another milk sample was collected from each of the animals. These samples were subjected to microbiological analyses and used as a control of the treatment's efficacy.

Treatment Efficacy Assessment Criteria

The efficacy of the treatment was assessed on the basis of a bacteriological examination. For the purpose of the study, a treatment was regarded as having been successful when the result of the bacteriological examination was negative for *S. aureus*.

Results

In Table 1 the distribution of cows in relation to the number of *S. aureus* infected udder quarters is presented. In 57 % (n = 21) of the selected cows only one udder quarter was infected. In 9 cows the infection was present in two, and in 6 cows in three udder quarters. There was only one case where all four quarters were infected.

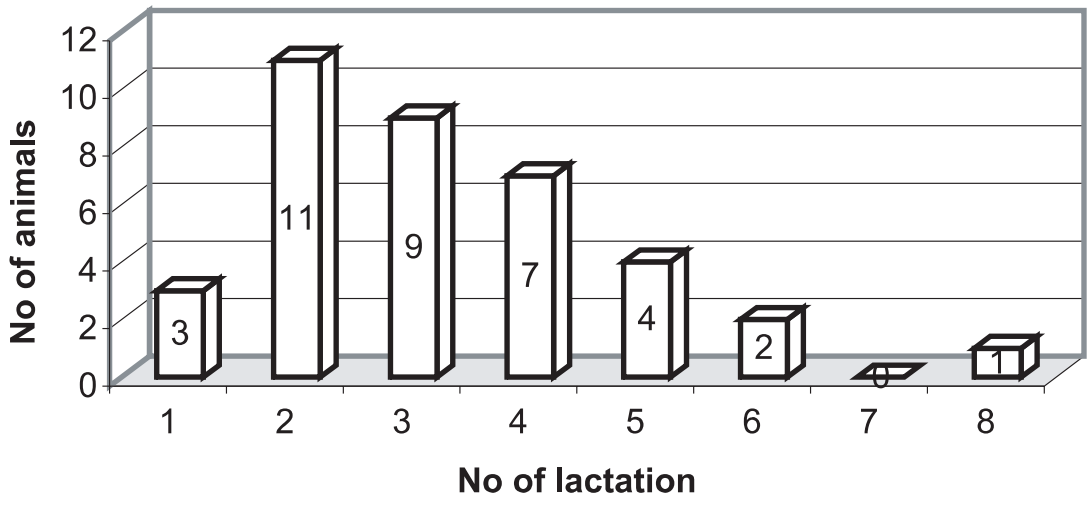
In Table 2 the results of the treatment are pre-

Table 1: Distribution of animals in relation to the number of infected udder quarters before treatment

No. of infected quarters per animal	1	2	3	4	TOTAL
No. of animals	21	9	6	1	37
No. of infected quarters	21	18	18	4	61

Table 2: Distribution of bacteriologically negative (successfully treated) animals in relation to the number of infected udder quarters before treatment

No. of infected quarters per animal	1 n = 21	2 n = 9	3 n = 6	4 n = 1	TOTAL	Bacteriological cure rate
No. of cured animals	13	5	1	0	19	51.3
No. of cured quarters	14	13	9	0	36	59.0
% of cured animals	61.9	55.5	16.7	0		
% of cured quarters quarters m.g	66.6	72.2	50.0	0		



Graph 1: Distribution of infected cows per lactation

sented in relation to the number of initially infected udder quarters. In our study we found a significant correlation between the number of successfully treated animals and the number of infected quarters. However, the difference in the percentages of successfully treated udder quarters was less significant.

Discussion

Synulox® was used in the treatment of 37 animals with one or more mammary glands infected by *S. aureus.*

The treatment protocol described above was selected in accordance with the claims of most authors that an efficient and effective treatment of subclinical and clinical mastitis caused by *S. aureus* takes at least 3 to 5 days (9.10).

The amoxycillin-clavulanic acid combination in the therapy was selected due to the high probability of the presence of ß-lactamase-positive strains *of S. aureus* in the infected animals (5, 6, 7).

There were differing opinions amongst authors regarding the best number of control samples to collect and when to collect them (7, 9, 10), hence, given our circumstances, we settled on one sample collection 14 days following the first application of the drug.

While the overall level of success using this bacteriological treatment (51.3 %) does not deviate substantially from claims made in other publications, it is clearly among the more successful methods, particularly as only subclinical types of bovine mastitis were treated (2, 7, 9, 10). The level of success that was achieved in treating animals with only one infected mammary quarter

(61.9 %) was very promising as they represented the majority (56.7 %) of all the treated animals. The comparative success of the treatment in our case is slightly diminished by the fact that in certain cases (n = 4), despite a successful bacteriological cure, the SCC did not fall below the level set down in the regulations governing the health and hygiene of milk (< 400,000 cells/ml).

While most of the infected animals were in their 2nd or 3rd lactation, which represents a lower average compared to some authors (2), we could not confirm a link between the number of lactations and the efficacy of the treatment.

Given our results we believe that the use of a combination of amoxycillin and clavulanic acid lived up to our expectations, and that the therapy of animals with only one or two infected mammary glands is sensible and economically justifiable. In our opinion however, it makes no sense to treat animals with three or four infected udder quarters irrespective of the type of therapy. In such cases culling should be seriously considered.

So far only a few studies of this type have been conducted in Slovenia. However, these previous studies considered both the clinical and subclinical forms of bovine mastitis together and therefore the results are not comparable (13, 14).

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ZDRAVLJENJE SUBKLINIČNIH STAFILOKOKNIH MASTITISOV

O. Podpečan, A. Pengov, U. Hrastnik

Povzetek: Namen raziskave je bil oceniti uspešnost zdravljenja subkliničnih mastitisov pri kravah molznicah, povzročenih z bakterijsko vrsto *Staphylococcus aureus*. *S. aureu*s je najpogosteje izolirana patogena bakterija pri živalih s subkliničnim mastitisom v ZDA in v Evropi. Podatki iz literature kažejo, da amoksicilin sam ni dovolj učinkovit proti S. aureus, v kombinaciji s klavulansko kislino pa se učinek zdravljenja bistveno izboljša. Okužene živali smo zdravili s preparatom Synulox®, ki vsebuje amoksicilin in klavulansko kislino. Preparat smo po navodilu proizvajalca aplicirali v mišico in v mlečno žlezo. Skupno je bilo zdravljenih 61 vimenskih četrti pri 37 kravah. Uspešnost bakteriološke ozdravitve je bila v povprečju 51,3 %, pri živalih z eno okuženo vimensko četrtjo pa 61,9 %, in sicer ob dejstvu, da so le-te predstavljale 56,7 % vseh živali v raziskavi. Glede na rezultate menimo, da je uporaba kombinacije amoksicila in klavulanske kisline upravičila naša pričakovanja in da je zdravljenje živali z eno ali dvema okuženima četrtma smiselno in ekonomsko opravičljivo.

Ključne besede: veterinarska medicina; mastitis - zdravljenje; *Staphylococcus aureus;* amoksicilin; klavulanska kislina