SUMMARY OF THE Ph-D THESIS

Epidemic surveillance and results regarding the diagnostic and therapy cows mastitis, on Bayern land, zone Allgäu from Germany

SCIENTIFIC COORDINATOR

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SUMMARY

Mastitis in female dairy cows in the area of Allgäu Germany is a significant issue and practitioners, farmers, milk processing factories and specialists working for the department laboratory make serious effort to prevent and fight this phenomenon.

The study was carried out in the period between 2003-2009, and focused on epidemiological aspects, on field diagnosis means, on laboratory examination confirmation, on the means of establishing the efficiency of medication used and on the assessment of the occurrence of the phenomenon of antibioresistance to the 10 most commonly used antibiotics.

The first part of the thesis covering 99 pages was drawn up based on bibliographic references (238 titles), and includes 9 chapters describing the following aspects: an introduction on the economic and sanitary importance of mastitis; the microbial agents involved in etiology (Gram-positive bacteria, Gram-negative bacteria, acid-alcohol resistant bacteria, microscopic fungi, unicellular algae, Chlamydia and viruses); the specific and non-specific mechanisms of the mammary gland defence; epidemiological aspects of mastitis; anatomical and clinical aspects and their presentation based on the clinical manifestations (serous, catarrhal, purulent, haemorrhagic, gangrenous) and depending on etiology (staphylococcic, streptococcic, pyobacillary, colibacillary, mycoplasmic, protothecal and mycotic); the field and laboratory diagnosis methodology; aspects regarding immunoprophylaxis and the principles of prevention and fight against this phenomenon.

The second part of the thesis covering 192 pages includes the results of my own research and is structured on 10 chapters describing the following investigations: the purpose and objectives of the thesis, the epidemiological aspects of mastitis in the studied farms, the field and laboratory diagnosis methodology, the aspects of the therapy applied in farms, the negative reflection of the errors of treatment on the farm economy, the evolution of the phenomenon of antibioresistance to the 10 most commonly used antibiotics, aspects of milking and its implications in mastitis, strategies of prophylaxis and fight against this phenomenon. The thesis concludes with the chapters dedicated to the general discussion of results, the final chapters and the bibliography, as well as with...
the annexes including the summary in Romanian and English, the lists and tables included in the thesis, the list of abbreviations, the list of the published works.

80% of the cows from the farms that are part of the study belong to the German brown cow, and the rest are Holstein and Flekvieh. Breeding certain cow breeds depends on the owner’s request, influenced in its turn by the focus laid on milk, or meat production or on both, (that is to say meat-milk production). Mastitis was identified in all categories of cows (lactating and in mammary repose). The total number of cases of mastitis registered over a period of three years: 285 cases in 2003, 321 cases in 2004 and 199 in 2005.

The epidemiological analysis aimed at establishing the degree in which the mamma was affected, depending on the number of the quarters affected and on their anatomical position. In as far as the degree in which the mamma was affected is concerned, we could notice that in most cases (72,21%) it was only one quarter that was affected, as compared to 14,1% of the cases in which two quarters were affected , 6.47% of the cases in which three quarters were affected and 7.10% of the cases in which the entire mamma was affected. Regarding the anatomical position of the quarters affected, one could notice that it was the quarters on the right side that had been affected more often. There was a higher incidence in the case of the right posterior quarters (37,20%), as compared to the right anterior ones (24,21%). By comparison, the left posterior quarters had a lower incidence (21,57%) as compared to the left anterior ones which had been the least affected ones (17,0%). Posterior quarters were more commonly affected (29,38%) as compared to the anterior ones (20,6%). The right posterior quarters were the most commonly affected ones (37,20%) as compared to the left posterior ones, which were less affected (21,57%). There was also a difference in the way in which the anterior quarters had been affected, that is to say, the infection had affected more frequently the right anterior quarters (24,21%) as compared to the left anterior part (17,0%).

The analysis of the mastitis evolution over time reveals that its distribution varies from one month to anther and from year to another. In the period between 2003-2005, we noticed that mastitis had a similar incidence during the months of January to June. After a significant decrease in the month of February, there was a constant increase until June-July. This period (the months of June-July), was followed by a period in which the
evolution differed greatly from one year to another over the studied period, with a pronounced variability in the autumn-winter months. Thus, in the year 2003 there was a pronounced decrease in the number of the cases until September, with a new increase until October, a decrease in November and a new increase in December. In 2004 there was a completely different evolution over the period between June and December, that is to say there was a very pronounced increase in the month of September, followed by a significant decrease until November, and a new increase until December. In 2005 the curve of affections had a decreasing evolution starting from June and until December, and in fact, this was also the year in which we recorded the smallest number of cases as compared to the previous two years. We can conclude that the evolution that was variable from the point of view of the distribution in time correlates with the intrinsic factors and especially with the extrinsic factors; thus, the more serious disturbances they recorded, the more they favoured the occurrence of the disease, by increasing the number of the contamination sources, the transmission and the increase of the infection pressure within shelters in the autumn-winter months.

For the epidemiologic surveillance of mastitis we devised a questionnaire which, in synthesis, includes the following data: identification of the farm, identification of the animal, aspects regarding maintenance, the milking system, identification of the period of occurrence, means of identifying mastitis, etiologic diagnosis and sensitivity to antibiogram, treatments applied and their results. The questionnaires allow for the gathering of data that can be statistically processed and which allow us to draw conclusions that reflect more realistically the intervention of the factors involved in the occurrence of mastitis, the efficient medication, the economic efficiency or other data that may reflect the vulnerable spots, especially in regard to the sources of contamination, to the deficiencies of milking and of the milking equipment.

The laboratory examinations results revealed that Gram positive bacteria were dominant when it came to the causes of diseases, especially the cocci belonging to the genera *Staphylococcus* and *Streptococcus*, while germs of other genera or Gram negative bacteria, such as *Escherichia coli* or other coliform were much more rarely identified (*Enterococcus, Arcanobacterium*).
Out of the 160 cases identified with mastitis in 2005 in the four farms that were part of the study, 211 bacteria strains were identified belonging to the following genera/strains: *Staphylococcus aureus* (105 strains), *Streptococcus ask*+ (61 strains), *Enterococcus* (30 strains), *Streptococcus ask*− (7 strains), coliform bacteria (6 strains), *Arcanobacterium* (2 strains). The isolated strains were responsible for the occurrence of mastitis in the following way: in 121 cases (75,62%) they belonged to one genus only, in 28 cases (17,50%) they belonged to two genera, in 10 cases (6,25%) they belonged to three genera and in 1 case (0,63%) they belonged to four genera.

Out of the 79 cases identified with mastitis in 2006 in the four farms that were part of the study, we identified 87 bacteria strains belonging to the following genera/strains: *Staphylococcus aureus* (45 strains), *Streptococcus ask*+ (30 strains), *Enterococcus* (5 strains), *Streptococcus ask*− (2 strains), coliform bacteria (3 strains). The isolated strains were responsible for the occurrence of mastitis in the following way: in 71 cases (89,87%) they belonged to one genus only, and in 8 cases (10,13%) they belonged to two genera.

Out of the 51 cases identified with mastitis in 2007 in the three farms that were part of the study, we identified 54 bacteria strains belonging to the following genera/species: *Staphylococcus aureus* (42 strains), *Streptococcus ask*+ (4 strains), coliform bacteria (8 strains). The isolated strains were responsible for the occurrence of mastitis in the following way: in 48 cases (94,12%) they belonged to one genus and in 3 cases (5,88%) they belonged to two genera.

Out of the 125 cases identified with mastitis in 2009 in the six farms that were part of the study, we identified 129 bacteria strains belonging to the following genera/species: *Streptococcus agalactiae* (56 strains), *Staphylococcus aureus* (38 strains), *Streptococcus ask*+ (24 strains), *Streptococcus ask*− (9 strains), *Streptococcus haemoliticus* (2 strains). The isolated strains were responsible for the occurrence of mastitis in the following way: in 121 cases (96,8%) they belonged to one genus, in 4 cases (3,2%) to two genera.

Monofactorial-etiology mastitis seems to be dominant, and the microbial flora is represented especially by cocci belonging either to *Staphylococcus* (the dominant ones being the coagulaz-positive strains), or to *Streptococcus* (the dominant ones being the ask+ strains), properties that ensure them high pathogenity as well as relevance for
classification. Most strains belonging to *Staphylococcus* and *Streptococcus* were producing penicillinase (an enzyme belonging to the β-lactamase group), which explains both the lack of efficiency of the therapy applied in some cases, and the favouring of the occurrence of the antibioresistance phenomenon.

For an efficient treatment, we perform an antibiogram in all cases with mastitis in the practice laboratory equipped with the minimum required devices: milk sample centrifuge, swab sampling, Petri tubes and plates, common culture media or antibiograms (Mueller-Hinton), and, if necessary, glucose or thermostat blood, disks for antibiuograms (microtablets) dispenser, classification tables. I would like to underline the fact that antibiograms offer very useful and rapid information that has allowed me to apply treatment based on the information they supplied. If necessary, I also perform microscopic examinations (bacterioscopic) which, depending on the form, group and tinctorial affinity, allow me to form an informed opinion on the aetiology which, in turn, allows me to establish therapy, prevention and fighting measures at farm level.

When devising the treatment formula, we have taken into consideration the evolution form of mastitis (clinic or subclinic), the animal’s general health condition, the period of evolution (lactating or in mammary repose), based on which the treatment was applied locally and generally, or, in many cases, only locally. When choosing the antibiotic we have taken into consideration the result of the antiobiogram, its bio-availability, its spreading to the mammary gland, the half-life period. We have paid special attention to the duration of its after-effect in milk (latency), an aspect of vital importance to the farmer, who must know this with precision, as during this time, milk cannot be traded. If this is not taken into consideration, then the farmer may be severely punished by the milk processing farm, which may even refuse to accept the milk supplied by the farmer in question. Within this context, we must also underline the fact that in order to ensure maximum efficiency to the treatment established, the dose must be very well complied with, as well as the interval between administration of the doses, the treatment length, as well as support of the antimicrobial action of the antibiotic, through an adequate support medication. If the local treatment is carried out by the owner (intramammary administration using injectors), then this is trained in order to apply it
correctly, but also with regard to the risks that might come up should they fail to comply with the protocol suggested by the veterinarian.

When applying the treatment, we have used many conditioned intramammary administration injectors, such as: Mastitar forte, Orbetin, Cobactan, Albiotic, Gelstamp, Rilexine, Neoclocx, Ubrolexin, Erytrisol, although others have also been used occasionally; the choice was generally dictated by the result of an antibiogram, but also depending on the stock available at that particular time and on previous efficiency.

At the same time farmers are informed that errors in the application of the treatment may lead to economic loss due to the impossibility of the milk processing factories to process the milk, as a result of the antibiotic’s presence in it, as well as to higher costs due to inefficient medication (under inhibitory dose) and to increases of the risk of selecting resistant strains. Such a situation was encountered in the F. H. farm, where, due to a lack of compliance with the treatment protocol, we could notice an increase in the number of cases over a period of two months, a diversification of the microbial flora involved in the aetiology and economic loss resulting from a prolongation of the half-way period (latency of the antibiotic), during which milk production could not be valorised.

In order to support the programmes fighting against mastitis occurrence, bacteriologic control of the cows in the entire farm is required whenever necessary or upon notification from the milk processing factories, in order to identify bacterial flora and to perform antibiograms, examinations carried out by the regional Laboratory. In this sense, a number of 764 isolated strains from cows suffering from various forms of mastitis and from various farms of the practice area have been tested using an antibiogram, the testing being carried out for the following antibiotics (microtablets): Ampicilline, Trimethoprim-sulfa, Cobactan, Albiotic, Rilexide, Peracef, Procain-Penicilline and Clocxacillin.

Ampiciline proved to be an efficient antibiotic. Out of a total of the strains examined, 588 strains (76,96%) proved to be responsive to it. Over the period studied strains sensitivity has maintained at high levels: 71,31% in 2003; 78,65% in 2004 and 74,26% in 2005.
Trimethosulpha manifested variable efficiency. 342 strains (44,76%) proved to be responsive. During the study period we could notice an increase in the sensitivity of the strains from one year to the other: 24,35% in 2003, 46,53% in 2004 and 58,42% in 2005. Consequently, it can be used for treatments.

Cobactan, an antibiotic containing cefquinome, belonging to the cephalosporin group, has proved to be a constantly efficient product. 527 (68,98%) proved to be sensitive to it. During the study period, the sensitivity of the strains maintained at appropriate values from one year to the other, with a slightly decreasing tendency: 69,57% in 2003, 69,52% in 2004 and 65,35% in 2005.

Albiotic is an antibiotic containing neomycine and lincomycin, that also proved to be efficient. Out of the total number of strains tested, 503 (65,84%) proved to be sensitive to it. During the study period, the sensitivity of the strains had an increasing tendency from one year to another: 58,26% in 2003, 65,69% in 2004 and 75,26% in 2005. Nevertheless, we feel that Albiotic remains an elective medication when it comes to the treatment of mastitis.

Rilexine, an antibiotic containing cephalexin also proved to be efficient, as 413 (54,06%) strains proved to be sensitive to it. During the study years, the sensitivity of the strains recorded similar values over the period in question: 58,26% in 2003, 52,19% in 2004 and 59,41% in 2005. Nevertheless, Rilexine remains an elective product in the treatment of mastitis.

Peracef, an antibiotic containing cephoperazone also proved to be an efficient product, as 431 (56,41%) strains turned out to be sensitive to it. During the study years, the sensitivity of the strains recorded similar values with an increasing tendency: 52,17% in 2003, 56,75% in 2004 and 59,49% in 2005. This antibiotic may also be considered to be an elective product in the treatment of mastitis.

Procain-penicilline proved to be less efficient, as only 169 (22,12%) strains proved to be sensitive to it. During the study years, the sensitivity of the strains recorded a decrease over time: 47,83% in 2003, 17,88% in 2004 and 15,84% in 2005. One can notice the dominance of the resistance phenomenon in the case of 532 strains (69,63%), with a marked increase over time: 48,69% in 2003, 72,63% in 2004 and 77,23% in 2005. In this context, the use of procain-penicilline as such or in the form of drugs must only be
used with extreme caution when it comes to the treatment of mastitis and only after an
antibiogram has been performed.

Clocxacillin is semi-synthetic penicillin and proved to be inefficient, as only 178
strains (22,12%) turned out to be sensitive to it. During the study years the sensitivity of
the strains recorded a marked decrease over time: 45,22% in 2003, 18,43% in 2004 and
24,75% in 2005. In the case of clocxacillin one can also notice the predominance of the
phenomenon antibioresistance, in the case of 525 strains (68,72%), the phenomenon
recording a marked increase over time: 49,56% in 2003, 71,9% in 2004 and 73,27% in
2005, which is why it must be used with caution.

It must be known that most bacteria involved in infectious syndromes, including
mastitis, have become resistant modifying their sensitivity in various degrees, which
makes antibiogram a crucial testing method when it comes to the sensitivity of the
microbial strains to the antibiotics used in therapy, salutary in the prevention of the
selection of antibioresistant strains.

To benefit from the efficiency of the antibiotics, these must be used after their
sensitivity has been tested with the use of an antibiogram. This is the reason why I always
perform this particular examination my practice, which helps me a great deal in
establishing a treatment that is both efficient resulting in the cure of the animal, and
economic - presupposing less expenses with medication and a more effective valorisation
of the milk, consisting in a reduction of the half-time period.

The thesis ends with well systematized conclusions that synthesise all the
objectives set. The bibliography is complex, comprising numerous works and syntheses
on mastitis, discussing and analyzing the various aspects of mastitis both from the point
of view of the animal’s health, and from that of its implications to public health.

The thesis also includes 29 tables and 84 figures (photos and graphs) judiciously
conceived and which reflect the various aspects of the phenomena studied.