31 WORLD BUIATRICS CONGRESS (WBC)

Submission date 23/02/2022

Submission time 2:22 PM

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Summary	
Reference number	1314
Title	Treatment of clinical mastitis: intramammary or in combination with parenteral administration of penicillin?
Topics	09. Udder Health and Mastitis
Presentation preference	ORAL
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Keywords	mastitis, treatment, intramammary, cure
Text	

Objectives

Antibiotics prescribed for dairy cows in Denmark are mainly penicillin products for parenteral mastitis treatment (DANMAP, 2020). However, it may be possible to reduce the amount of penicillin used, if intramammary (IMM) treatment is sufficient as an alternative to parenteral or combined IMM and parenteral treatment which is the first choice for Danish veterinarians (Wilm et al., 2021). This study aimed to investigate if IMM administration of penicillin was non-inferior to IMM combined with parenteral administration of penicillin regarding bacteriological cure of non-severe clinical mastitis cases caused by Gram-positive bacteria.

Materials and methods

The study was carried out as a non-inferiority longitudinal randomized clinical trial in 12 Danish dairy herds from May 2020 to June 2021. The treatment protocols evaluated were **A**) IMM treatment for three days, and **B**) both IMM and parenteral treatment for three days. The IMM treatment comprised 600,000 IE benzylpenicillinprocain administered in the affected quarter once per day for three days. The parenteral treatment comprised 15,000 IE penethamatehydroiodid/kg body weight administered in the muscle (IM) once per day for three days. Treatment A and B were randomly assigned to cows with non-severe clinical mastitis caused by a Gram-positive bacteria. The farm personnel used an on-farm test to get an indication of this classification (Gram-positive/negative) within the first day of symptoms and before the onset of antibiotic treatment. In the meantime, all clinical mastitis cases were treated with ketoprofen in a dose of 3 mg/kg body weight. Subsequently, the quarter milk samples were cultured and pathogens were identified using MALDI-TOF. A case was included if one or two bacterial species were identified, and at least one of those were Gram-positive. Bacteriological cure was evaluated on two follow-up samples, two and three weeks after ended treatment. A case was deemed to be cured if the pathogens present in the clinical sample were not detected in any of the follow-up samples. A non-inferiority analysis was carried out with a margin of 15%. **Results**

Of almost 1,800 clinical mastitis cases registered, 347 were eligible for evaluation of bacteriological cure based on treatment A or B. The results showed that the cure rate depended on the pathogen. The overall cure for treatment A was 76% [95% CI: 69.2-82.5] and the overall cure for treatment B was 83% [95% CI: 76.8-88.3]. Treatment A was not non-inferior to treatment B with a margin of 15% across all bacterial species. However, the majority (183) of included mastitis cases involved *Streptococcus uberis* infection, either alone or in combination with another species. When including only these cases in the analysis, the cure rate for treatment A was 83% [95% CI: 74.1-90.1], and the cure rate for treatment B was 81% [95% CI: 70.9-88.3]. Thus, treatment A was non-inferior to treatment B for cases with

Streptococcus uberis.

Conclusions

Penicillin administered IMM for three days is no worse than penicillin administered both IMM and parenteral for three days when it comes to bacteriological cure of non-severe clinical mastitis caused by *Streptococcus uberis*.

Implementation of this result in Danish dairy herds can reduce antibiotics used for clinical mastitis treatment. However, the reduced treatment was not found non-inferior across all bacteria species. This underlines the importance of accurate mastitis diagnostics and the need to adjust treatment protocols to the herd's specific mastitis pathogens, in order to facilitate prudent use of antibiotics.

The research project was funded by the Danish Milk Levy Foundation

References

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Wilm, J., L. Svennesen, E.Ø. Eriksen, T. Halasa, and V. Krömker. 2021. Veterinary Treatment Approach and Antibiotic Usage for Clinical Mastitis in Danish Dairy Herds. Antibiotics 10:189. doi:10.3390/antibiotics10020189.

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