

BOVINE MASTITIS: EPIDEMIOLOGICAL, CLINICAL AND ETIOLOGICAL STUDY IN A SAUDI ARABIAN LARGE DAIRY FARM

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Summary

Fadlelmula, A., A. M. Al Dughaym, G. E. Mohamed, M. K. Al Deib & A. J. Al Zubaidy, 2009. Bovine mastitis: Epidemiological, clinical and etiological study in a Saudi Arabian large dairy farm. *Bulg. J. Vet. Med.*, 12, No 3, 199–206.

The epidemiological, etiological and clinical aspects of mastitis in a Saudi Arabian dairy farm with a capacity of 11,200 dairy cows were investigated for a period of 60 days in winter. The disease incidence was 1.8 cases per 100 cows/month. The first month of lactation displayed the highest incidence of mastitis (62.7%), while the late stage of lactation showed the lowest incidence (11.2%). The disease incidence increased with increasing age and lactation number. Repeated occurrence of mastitis was 65.1% among cows with a significant decrease in milk yield ($P < 0.05$). Mild and acute forms were the only types of mastitis diagnosed during the study period (59.7% and 40.3% respectively). In the mild form, udder temperature was normal in 97.0%, body temperature was increased in 0.9% and general dullness was present in 1.3% of cases. The acute form was characterized by hot udder (63.9%), increased body temperature (35.4%) and dullness (37.0%) of cases. The most predominant bacterial isolates were *Streptococcus uberis* (28.8%), *Escherichia coli* (18.5%), *Streptococcus dysgalactiae* (10.8%) and *Staphylococcus aureus* (9.8%). Other bacteria and fungi were isolated in a range of 0.3 to 3.8%. This is the first report of bovine mycotic mastitis from the Kingdom of Saudi Arabia. Medicinal treatment was effective in 95.3% of acute cases and 97.8% of mild cases. In 2.2% of the cases, infection proceeded to chronicity and culling. Irresponsiveness to treatment and hence culling was observed most commonly in infections of *Staph. aureus* and *E. coli*.

Key words: dairy cattle, epidemiology, etiology, management, mastitis

INTRODUCTION

Bovine mastitis is an important disease that affects animal health and production together with public health. It has substantial economical implications on the dairy industry and is still a major challenge despite the widespread implementation of mastitis control strategies.

Many bacterial and fungal species were incriminated as causative agents of bovine mastitis. The most common bacterial isolates include: *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, *Arcanobacterium pyogenes*, *Mycoplasma bovis* and *M. californicum* (Bramley, 1978; 1982). Fungal isolates include

Aspergillus spp., *Candida* spp., *Cryptococcus neoformans* (Ainsworth & Austwick, 1973; Richard *et al.*, 1980; Elad *et al.*, 1995).

Epidemiological data are essential to understand the magnitude of the problem in a particular farm. The prevalence of clinical mastitis was reported to be 52.4 cases/100 cows in Uruguay (Giannechini *et al.*, 2002). In Finland, the prevalence of mastitis was 38% in 1995 and 31% in 2001 (Pitkala *et al.*, 2004).

Treatment of mastitis aims at removing pathogens from an infected quarter and restoration of milk's normal composition. The degree of response depends on the causative agent and the timely initiation of treatment.

Published reports on bovine mastitis from the Kingdom of Saudi Arabia (KSA) are few. Therefore, the present study was undertaken to determine the epidemiology, etiology, clinical picture and management of the disease in a large commercial dairy farm in the Eastern Region of the KSA during the winter.

MATERIALS AND METHODS

Animals

The study was conducted in a large commercial dairy farm in the Eastern Region of the KSA rearing 11,200 primiparous or multiparous dairy cows from the Holstein breed. They are kept in yards with sand as bedding of a capacity of 120 animals per house. Animals were raised using intensive husbandry practices utilizing modern technology such as cow cooling and advanced feeding systems. The grouping of cows depended on the milk production. Each unit had special calving pens. Sick and lame animals were segregated in separate pens.

Cattle were zero-grazed and fed with a total mixed ration according to performance of milk productivity in each group. Animals were machine-milked up to three times a day; and milking was carried out under strict hygienic measures. The average daily yield per cow was 32 L. The milk yield of each cow was measured every two weeks. Somatic cell count (SCC) on bulked milk from the farm was done every two weeks. Post-milking treatment of teat with iodine was practiced. Dry-cow therapy using AI-dry (Dawa Pharmaceuticals, Jordan) was applied to each cow.

The study was conducted for 60 days during winter. All cases of mastitis in the farm during this period were included in the study. Epidemiological data and case history of mastitic cows were recorded. Milk specimens for microbiological investigations were collected in sterile screw-capped containers and sent in an ice-box to the laboratory. Each specimen was cultured in duplicate onto 5% sheep blood agar, MacConkey's agar (Oxoid), Hayflick modified medium and Sabouraud's dextrose agar (Oxoid). In addition, swabs were collected from the cows' bedding (20 specimens), milk tanks (n=10), the milking hall ground (n=18) and udder skin and teat (n=30) for screening of bacteria and fungi.

Treatment and treatment efficacy evaluation

A thorough clinical examination on mastitic cows was performed. California mastitis test was performed on milk samples from suspected cows. Medicinal treatment was applied to mastitic cows according to clinical findings, California mastitis test and sometimes guided by antimicrobial sensitivity tests.

The treatment consisted in: intramammary infusions with procaine ben-

zylpenicillin (300000 I.U.) + kanamycin sulphate acid (100 mg) + prednisolone (20 mg) (Penikan P300, Kela Laboratories, Belgium), external application of ampicillin + cloxacillin ointment (Lactoclox, Norbrook Laboratories, (U.K.); gentamicin sulphate ointment (Gentamast, Dawa Pharmaceutical, Jordan), drugs for topical use: Udderoid ointment (Dawa Pharmaceuticals, Jordan) containing eucalyptus, iodine, phenol and camphor for chronic mastitis; Cetrimide ointment for subacute and acute mastitis. Intramammary infusions and drugs for topical application were applied once daily for three days. Drugs for intramuscular injection: procaine penicillin (200 mg) + streptomycin sulphate (200 mg) were administered for three days.

A group of 66 cows with *E. coli* mastitis were selected in a trial to determine the effect of treatment. All cows were treated with intramammary infusions of gentamicin sulphate (500 mg/injection repeated every 12 h for three days). Thirty of them were also treated with the non-steroidal anti-inflammatory drug ketoprofen (Anafen, Meril Canada Inc.) at 100 mg/mL given intramuscularly at a dose of 1.5 ml/50 kg once a day for three days. Treatment response and outcome were assessed clinically and microbiologically.

Microbiological cure was defined as not having the same bacterial species isolated from the quarter milk samples taken at two and four weeks post-treatment as in the samples taken before treatment.

Univariate analysis of variance was carried out to compare groups.

RESULTS

Epidemiology

Clinical mastitis was diagnosed in 398 out of 11,200 cows giving a monthly inci-

dence of 1.8%. The mean SCC in bulked milk during the study period was 2.73×10^6 cells/mL.

Most mastitis cases were among cows in their first months of lactation (62.7%), 26.1% were in their mid lactation and 11.2% were in the late stages of lactation. With regard to age, it appeared that disease incidence increased with increasing age (Fig. 1). Also, the number of calvings influenced the number of times the animal contracted the disease. As lactation number increased, incidence of mastitis also increased (Fig. 2).

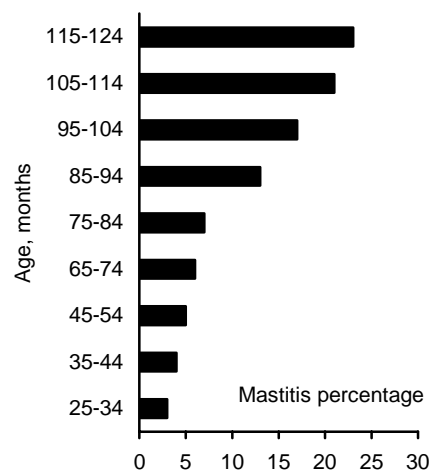


Fig. 1. Effect of animal age on the incidence of mastitis in the studied large dairy farm.

A total of 139 mastitic cows have not experienced mastitis before (34.9%), while 259 cows have encountered the disease at least once (65.1%). There was a difference between the average milk yield from cows that had previous mastitis (26.13 L) and those which had not (29.43 L; $P < 0.05$). The difference in milk production between primiparous and multiparous cows was not statistically significant.

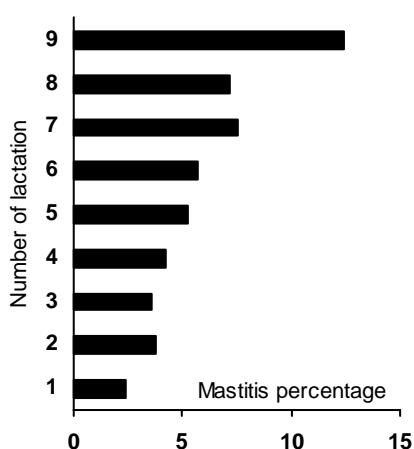


Fig. 2. Effect of lactation number on the incidence of mastitis in the studied large dairy farm.

Clinical studies

Two types of mastitis, mild and acute, were diagnosed during the study period. The mild type was diagnosed in 238 (59.8%) and acute mastitis in 160 cases (40.2%). To compare general clinical manifestations of the types of mastitis, udder temperature, body temperature and the general health condition were assessed.

The effect of mastitis types on udder temperature is shown on Table 1. Of the 398 cases included, body temperature was normal in 101 cases (63.1%) and high in 59 (36.9%) in the acute type. In the mild type, 236 cases were normal (99.2%) and 2 cases (0.8%) had high body temperature. In the acute type, 109 cows (68.1%) appeared in normal general health and 51 (31.9%) were dull. In the mild type, 235 (98.7%) showed normal appearance and 3 (1.3%) were dull. No animal showed signs of systemic disorder, while no animal reported dead during the study period.

The treatment response among the types of mastitis is displayed in Table 2. The response was found to be favourable in 88.8% of the acute cases and 95.4% of the mild cases. The response was bad in 10% of the acute and 3.8% of the mild cases; 1.3% of the acute and 0.8% of the mild cases were culled.

Efficacy of treatment

In the group of 36 cows which were treated with antibiotics alone, 25 responded favourably to the treatment with a recovery rate of 69.4%. The second group of 30 cows, treated with antibiotic + antiinflammatory drug, 27 responded

Table 1. Effect of the types of mastitis on udder and body temperatures and on general condition of mastitic cows in a large dairy farm

| | | Number of cows with: | | Total number |
|-------------------|--------|----------------------|----------------|--------------|
| | | Mild mastitis | Acute mastitis | |
| Udder temperature | cold | 4 | 7 | 11 |
| | normal | 230 | 51 | 281 |
| | hot | 4 | 102 | 106 |
| Body temperature | normal | 236 | 101 | 337 |
| | high | 2 | 59 | 61 |
| General condition | normal | 235 | 109 | 344 |
| | dull | 3 | 51 | 54 |

Table 2. Response of mastitic cows to treatment of the different types of mastitis in a large dairy farm

| Type of mastitis | Response | | | |
|------------------|------------|-----|--------|-------|
| | Favourable | Bad | Culled | Total |
| Acute | 142 | 16 | 2 | 160 |
| Mild | 227 | 9 | 2 | 238 |
| Total | 369 | 25 | 4 | 398 |

Table 3. Bacterial and fungal isolates from cases of bovine clinical mastitis in a large dairy farm in the Kingdom of Saudi Arabia

| Isolated species | Frequency | Percentage |
|-----------------------------------|-----------|------------|
| <i>Streptococcus uberis</i> | 115 | 28.9 |
| <i>Escherichia coli</i> | 74 | 18.6 |
| <i>Streptococcus dysgalactiae</i> | 43 | 10.8 |
| <i>Staphylococcus aureus</i> | 39 | 9.8 |
| <i>Streptococcus agalactiae</i> | 15 | 3.8 |
| Coagulase-negative staphylococci | 14 | 3.5 |
| <i>Corynebacterium</i> spp. | 11 | 2.8 |
| <i>Klebsiella oxytoca</i> | 8 | 2.0 |
| <i>Pseudomonas aeruginosa</i> | 6 | 1.5 |
| <i>Shigella</i> spp. | 4 | 1.0 |
| <i>Enterobacter</i> spp. | 3 | 0.8 |
| <i>Proteus mirabilis</i> | 2 | 0.5 |
| <i>Candida krusei</i> | 4 | 1.0 |
| <i>C. parapsilosis</i> | 1 | 0.3 |
| <i>Trichosporon asahii</i> | 1 | 0.3 |
| <i>Aspergillus fumigatus</i> | 1 | 0.3 |
| No growth | 57 | 14.3 |
| Total | 398 | 100.0 |

favourably to treatment with a recovery rate of 90.0%.

The most frequent etiological agents were *S. uberis* (28.8%), *E. coli* (18.5%), *S. dysgalactiae* (10.8%) and *St. aureus* (9.8%). The most predominant fungal isolate was *Candida krusei* (1.0%). *Mycoplasma* spp. were not reported from these animals. No growth was found in 14.3% of cultures (Table 3). Screening

for *Mycoplasma* spp. from milk of the milk tank was negative.

DISCUSSION

The monthly incidence of mastitis in the farm was found to be 1.8% giving an annual incidence of 21.6%. This figure lies within a range reported for the annual herd-year-incidence which varied from 4.2 to 26.8 cases per 100 cows/year

(Giannechini *et al.*, 2002). Most of the cases in the present study were detected in the early stages of lactation.

Age and lactation number affected the frequency of mastitis in the present study. Disease incidence increased with age and lactation number. Culling and consequent herd replacement could have initially maintained high producing cows and affected the number of animals in the old-age groups. Thus, this group faced stress of calving and lactation together with the fact that high-producing cows are more susceptible to udder infection; the relatively long in-milking time and distended udders being more exposed to environmental factors and pathogens. About $\frac{2}{3}$ of studied cows have encountered mastitis at least once before, while $\frac{1}{3}$ of animals have not, indicating that a previous infection may predispose to a second attack. Previous infection also led to a drop in milk yield as there was a significant difference ($P < 0.05$) between previously infected cases and not-infected ones. This adds evidence about the economic importance of the disease in this farm. In another study, total milk loss for second-plus lactation cows was estimated as 1181 kg per lactation (Wilson *et al.*, 2004).

Effect of previous infection on the reproductive performance of cows was investigated. Statistical analysis revealed that previous infection had no effect on its fertility condition. On the other hand, a high percentage (82.3%) of animals proved fertile among the previously-infected group. This could be attributed to the fact that fertile animals may be more exposed to infection by entering into more lactations with the stress of the udder activity and milking procedures.

Microbiological investigation revealed that 58.3% of the mastitis cases were caused by *S. uberis*, *E. coli* and *S. dysgalactiae*. These species are described as

environmental causes of mastitis and *S. uberis* and *E. coli* are associated with faeces and animal bedding (Bramley, 1982; Harmon *et al.*, 1992). The high frequency of *Streptococcus* spp. may be explained by a study that recorded a high count of *Streptococcus* spp. compared to coliforms in recycled sand used for bedding (Krisztula *et al.*, 2005). Bedding can be a source of contamination for udders and teats which presents animals with a continuous challenge as animals are kept inside pens. These findings indicated that during the study period in winter, environmental mastitis prevailed. In temperate climates where animals are kept indoors in winter and turned out to pasture in spring and summer, contagious mastitis prevails during winter. Animals, in the present study, are raised using intensive husbandry practices and zero-grazed thus are in contact with each other all the year round; hence, one expects contagious mastitis to prevail. Such a hypothesis is now under investigation.

During the present study, only mild and acute mastitis was diagnosed, with no gangrenous mastitis. Most of the cases were mild (59.8%), while the acute type was diagnosed in 40.2%. The acute form caused 96.2% of mastitic udders to be hot in comparison to 3.8% caused by the mild form. In the effect of mastitis on body temperature, a two-tailed correlation between the two types of mastitis and body temperature showed a negative correlation (coefficient of correlation -0.478 , significant at $P < 0.01$). It means that if mastitis is acute, then the body temperature is high and vice versa. In 32% and 1.3% of acute and mild cases respectively, animals appeared dull. Summarizing the signs of fever and animal general health condition, it appears that, at least in some cases, the acute form induced a systemic reaction. It is worth

mentioning that out of *E. coli* isolates, 54.1% caused mild infection and 45.9% caused acute disease contradicting the general view that *E. coli* is always associated with acute infection. In comparison to other isolates, *E. coli* caused 21.4% of all acute cases while *S. uberis* caused 32.1%, *St. aureus* 10.1% and *S. dysgalactiae* 9.8% of acute mastitis. Mycotic mastitis was also considered during the present study. Fungi (predominantly yeasts) were isolated from 1.8% of mastitis. *Candida krusei* was the most frequent causative agent. Similar findings were reported by other investigators (Richard *et al.*, 1980; Costa *et al.*, 1993; Aalbaek *et al.*, 1994). *C. krusei* caused 1.3% of all acute mastitis while *A. fumigatus* and *T. asahii* caused 1.2% of cases. In all cases there was spontaneous recovery. This is the first report of bovine mycotic mastitis from the KSA. The yeast-like fungus *T. asahii*, has not been reported previously as a cause of mastitis. It could possibly be an opportunistic pathogen and needs further attention when identifying mastitis etiologic agents. Treatment with intramammary infusions of antibacterial antibiotics may predispose mycotic mastitis. In the present study, antimicrobial agents were administered intramammary or systemically for treatment (Table 2). A fair percentage responded favourably; some showed bad response which necessitated administration of a new combination of antimicrobial agents when infection proceeded to chronicity and culling. In the experiment conducted to assess treatment efficacy, gentamicin was selected because a high percentage of *E. coli* isolates were found to be sensitive. Similar findings about *E. coli* isolates' sensitivity to gentamicin was reported (Lehtolainen *et al.*, 2003). In the present study, the confounding effect of an anti-inflammatory drug was clearly demonstrated in a high recovery

percentage (90.0%) in the group treated with the antibiotic + an anti-inflammatory drug compared to the group treated with antibiotic only (69.4%).

Of the mastitic cows recommended for culling, 50% were due to *Staph. aureus*, 25% to *E. coli*. In 25% no etiological agents were recovered. It has been demonstrated that clinical mastitis significantly increased the risk of a cow being culled for a period of at least 2 months after any clinical mastitis case (Bar *et al.*, 2008). The high rate of culling due to *Staph. aureus* infection in the present study, points clearly to difficulty in management of mastitis caused by this species in the farm during the study period.

ACKNOWLEDGMENTS

We are grateful to King Abdul Aziz City for Science and Technology for the generous funding of the project AR-20-30. We wish to thank Mr. Abdul Latif Al Sager for his technical assistance.

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Paper received 28.08.2008; accepted for publication 13.01.2009

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