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# Clinical, bacteriological and epidemiological aspects of clinical mastitis in Israeli dairy herds

N.Y. Shpigel<sup>a,\*</sup>, M. Winkler<sup>b</sup>, G. Ziv<sup>b</sup>, A. Saran<sup>b</sup>

<sup>a</sup> *The Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, P.O.B. 12, Rehovot 76100, Israel*

<sup>b</sup> *The Kimron Veterinary Institute, Beit Dagan 50250, Israel*

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## Abstract

A 4-year retrospective study was performed to determine the clinical, bacteriological and epidemiological aspects of acute clinical mastitis in seven Israeli dairy herds. A total of 1124 clinical mastitis cases were detected by abnormal changes in the milk and udder with concurrent decrease of at least 25% in daily milk production. A total of 1190 quarters were affected with clinical mastitis in 1089 cows. The rear quarters had a higher incidence risk (64.7% of quarter cases) than the front quarters. The annual herd-year-incidence varied from 4.2 to 126.8 cases/100 cows/year. The whole-lactation incidence risk (LIR) was 20.8 per 100 lactations. LIR increased from the first to fifth lactation and then decreased. Most clinical mastitis cases were associated with coliform bacteria (60.2% of cases), environmental streptococci (18.6%), coagulase-negative staphylococci (8.7%) and samples from which no bacterial growth was detected (8.1%). Most cases of clinical mastitis occurred in the early stages of lactation, with 51.4% of all cases, 52.3% of coliform cases and 54.6% of environmental streptococci mastitis cases occurring during the first 4 months of lactation. The median days in milk at diagnosis was 118 days. The incidence was lower in the dry summer months. The ratio of peak to low incidence was 1.62 with a calculated peak incidence in January. © 1998 Elsevier Science B.V.

*Keywords:* Cattle, microbiological diseases; Mastitis

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## 1. Introduction

The implementation of effective specific control programs over the last decade resulted in the eradication of *Streptococcus agalactiae* and substantially reduced the

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\* Corresponding author.

incidence of *Staphylococcus aureus* subclinical mastitis in many well-managed herds. However, in some herds, acute clinical mastitis has become a major problem. In fact, clinical mastitis is one of the most common diseases of dairy cows in many herds (Wilesmith et al., 1986; Erskine et al., 1988; Gonzalez et al., 1990; Hogan et al., 1989). The incidence of clinical mastitis can vary between 5 to 110 cases/100 cows/year (Wilesmith et al., 1986; Hogan et al., 1989; Erskine et al., 1991). This situation is of great economic importance due to loss of milk production, culling and death of cows, cost of drugs and veterinary services and perhaps reduced reproductive efficiency (Cullor, 1990). The implementation of proper therapeutic and preventive measures depends on the appropriate knowledge of clinical, epidemiological and bacteriological aspects of clinical mastitis. To the best of our knowledge, reports dealing with large-scale studies of clinical mastitis relied upon farmer-observed disease incidence with little, none or selective (severe cases only) professional veterinary input at the stage of diagnosis and treatment. This might have created problems of case definition and/or reporting or submission bias. The practice area of The Ambulatory Clinic of The Koret School of Veterinary Medicine renders herd health and clinical services to high-producing dairy herds under intensive management. The intensive system of computer-controlled milking-parlour and herd management together with disease surveillance and data collection by veterinary professionals, offers a unique opportunity for accurate and reliable field studies.

The objectives of this study were to record the incidence of severe clinical mastitis associated with decreased milk production in seven Israeli dairy herds and to identify etiological and epidemiological factors affecting these cases.

## 2. Materials and methods

### 2.1. Dairies

Data on clinical mastitis from September 89 through December 93 in seven dairy herds located in the central part of Israel were analyzed retrospectively. The herds were within the practice area of the Ambulatory Clinic of the Koret School of Veterinary Medicine, which provided a complete herd-health service and the herds were visited every other day. All clinical, reproduction, production and management data were computer recorded by the herd manager and the attending veterinarian. These herds consisted of 50–300 milking cows (a total study population of about 1000 Israeli Holstein cows). In five of the herds, cows were milked three times a day and the average annual milk production ranged between 9000 to over 10000 kg per cow. Two herds were milking twice daily with an average annual milk production of about 8000 kg per cow. Cows were kept under a loose housing system and fed a total mixed ration. Dry cows were kept separately and fed high-quality wheat hay supplemented with lactating cows ration. Dry-cow therapy was not used with the exception of one herd where selective intramammary infusion was given to *Sta. aureus*-infected cows. Once a month, individual-cow and bulk-tank milk was sampled and analyzed for somatic-cell count by

the Central Laboratory for Milk Recording. A composite milk sample was obtained at least annually from every lactating cow and submitted for bacteriological examination. The annual herd-year-average somatic-cell counts varied from 350 000 to 700 000 cells/ml. The annual herd-year-average subclinical *Sta. aureus* infection rates varied from 0 to 37%. All herds were free of *S. agalactiae* during the study period.

## 2.2. Clinical mastitis case definition

Clinical mastitis was defined by the diagnosis of abnormal changes (acute, local, and systemic) in the body, udder, and milk, with concurrent decrease of at least 25% in daily milk production. Changes in the udder included pain, swelling, warmth, and abnormal appearance of milk (e.g. watery, clots, flakes or blood). Clinical mastitis was initially diagnosed by trained dairy employee by the routine use of computerized on-line milk conductivity system and the strip cup method to detect abnormal milk from the cow's udder quarter. All the cases were further examined and recorded by the attending veterinarian. Only lactating cows at least 2 days in milk were included in the present study. Cows with clinical mastitis as a sequel to teat injury or any udder trauma were not included.

The decrease in milk production on the day of diagnosis as well as return to production after treatment were retrospectively compared to the average milk production in the 10 days before diagnosis.

The outcome of every case was categorized into one of the following groups: recovered, cows that returned to at least 75% of the pre-mastitis daily milk production; blind quarter, cows in which lactation ceased in the affected quarter for the duration of the present lactation; culled, cows that died, were salvage slaughtered, culled or did not return to at least 75% of pre-mastitis daily milk production. A previously infected quarter was counted as having a new clinical infection when cow was categorized as recovered after the previous episode, regardless of the time elapsed between the episodes.

## 2.3. Bacteriological examination

Using aseptic technique, a milk sample was collected from the affected quarter(s) of all cases prior to treatment as recommended (Brown et al., 1982). Samples were immediately frozen at  $-5^{\circ}\text{C}$  and submitted for bacteriological examination within 2 days.

Milk samples were thawed at room temperature. A sterile, plastic disposable bacteriological loop was used to spread 0.03 ml of each milk sample into a freshly prepared blood–agar plate (Bacto–Agar, Difco Laboratory, Detroit, MI) containing 5% washed sheep red-blood cells, and into MacConkey agar (Difco). Several drops of  $\beta$ -haemolytic *Sta. aureus* and *S. agalactiae* overnight broth cultures were separately added to the blood–agar plates to permit reading the CAMP reaction of either streptococcal or CAMP-positive staphylococcal colonies on primary culture. Plates were incubated in air at  $37^{\circ}\text{C}$  and were examined for growth several times daily for the next few days. If growth did not appear within 7 days, plates were considered negative. Gram stain and

culture characteristics (i.e. colony morphology, pigmentation, aroma and haemolysis) were used for presumptive identification for all isolates.

All Staphylococci with a  $\beta$ -haemolytic pattern, a positive coagulase reaction in rabbit plasma and a positive CAMP reaction were presumed to be *Sta. aureus*. All coagulase-negative staphylococci (CNS) were further examined with the apiSTAPH system (apiSTAPH, Bio Merieux SA, Mary-l'Etoile, France). Species identification was performed using the APILAB Plus bacterial-identification software. We recorded all CNS as such without species identification. Streptococci were identified by haemolytic patterns, CAMP reaction and hydrolysis of esculin on esculin blood agar (sheep-blood agar with 0.05% esculin and 0.01% ferric citrate). All Streptococci were typed for Lancefield serological grouping (Phadebact Streptococcus Test, Pharmacia, Uppsala, Sweden).

Coliform bacteria and other Gram-negative bacilli were identified using culture characteristics on MacConkey agar, growth in triple sugar iron agar, urease, catalase, oxidase and indole production. Corynebacteria and *Actinomyces pyogenes* were identified using culture characteristics on blood agar, motility and catalase and urease production.

Bacterial suspensions were inoculated into Mueller–Hinton agar plates (Muller–Hinton Agar, Difco) and antimicrobial susceptibility tests were performed on all organisms by use of antimicrobial-impregnated disks (Dispens-O-Disc, Difco). The in vitro disc sensitivity tests were interpreted by the NCCLS standards (National Committee for Clinical Laboratory Standards, 1983). Determination of which colonies to consider as pathogens was based on colony numbers of specific organisms isolated pure

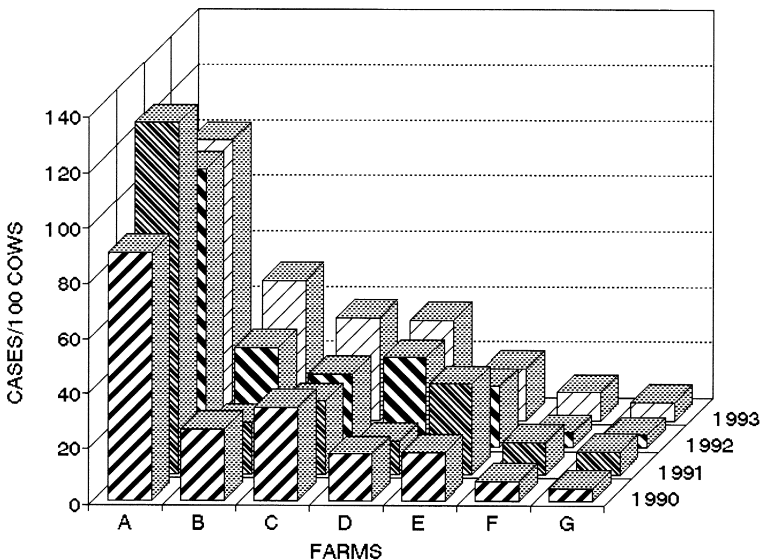


Fig. 1. Annual incidence risks of 1124 cases of clinical mastitis in Holstein dairy cows in seven Israeli herds in 1990–1993.

or with other colony types as described by the National Mastitis Council 1987 (Barnes-Pallesen et al., 1987).

#### 2.4. Statistical analysis

All episodes of clinical mastitis as defined above were included in the statistical analysis. Incidence risks of clinical mastitis were computed by dividing the number of occurrences of clinical mastitis during a defined period by average number of lactating cows during that period. The association between parity and clinical mastitis incidence was measured by ratio of risks and their significance was estimated by chi-square ( $\chi^2$ ) and the extended Mantel–Haenszel procedure (Breslow and Day, 1980). Data were stratified in the Mantel–Haenszel procedure on herds and type of cultured organism (defined as environmental streptococci, coliforms and all others) and the odds ratios (OR) and 95% confidence intervals (CI) were calculated. The Pearson  $\chi^2$  test was used to separately analyze the association of clinical mastitis quarter location with type of cultured organism or parity. The seasonal variation of clinical mastitis incidence was tested with Edward's test corrected for changing size of the population at risk in different months (Edwards, 1961). Significance was set at two-tailed 0.05 for all tests.

Table 1

Promotional prevalences of cultured microorganisms from quarter samples of 978 cows affected by clinical mastitis in seven Israeli Holstein dairy herds, 1989–1993

Organism	<i>n</i>	%
<i>Escherichia coli</i>	575	51.2
<i>S. dysgalactiae</i>	120	10.7
Coagulase-negative Staphylococci	86	7.7
Negative	79	7.0
<i>Sta. aureus</i>	72	6.4
<i>A. pyogenes</i>	55	4.9
<i>Streptococcus</i> spp.	38	3.4
<i>S. uberis</i>	21	1.9
<i>Corynebacterium</i> spp.	15	1.3
<i>Klebsiella</i> spp.	11	1.0
<i>C. pseudotuberculosis</i>	10	0.9
<i>Candida krusei</i>	7	0.6
<i>Enterococcus</i> spp.	7	0.6
<i>Enterobacter</i> spp.	6	0.5
<i>S. agalactiae</i>	5	0.4
Contaminated samples	4	0.4
Growth inhibitors	2	0.2
<i>Proteus</i> spp.	2	0.2
<i>Acinetobacter</i> spp.	2	0.2
<i>Pneumococcus</i> spp.	1	0.1
<i>Trichospora capitatum</i>	1	0.1
<i>Pseudomonas aeruginosa</i>	1	0.1
Unidentified yeast	1	0.1
<i>Prototheca</i> spp.	1	0.1
<i>Can. rugosa</i>	1	0.1
<i>Pasteurella</i> spp.	1	0.1

### 3. Results

During the 4-year study, 1124 cases of acute clinical mastitis were detected in lactating cows. The annual incidence of clinical mastitis varied widely in between herds and years (Fig. 1) from 4.2 to 126.8 cases/100 cows at risk per year. The average incidence risks for 1990–1993 were 23, 27.6, 25.6 and 30.7 cases/100 cows/year, respectively.

The lactational incidence risk (LIR) for the whole period was 20.8 cases per 100 lactations. The LIR of clinical mastitis increased from first to fifth lactation and then decreased. The LIR was 14.3, 19.6, 26.7, 27.4, 29.2, 22.3, 26.2 and 17.9 cases/100 cows in lactation number 1, 2, 3, 4, 5, 6, 7 and 8, respectively. After controlling for the farm and cultured organism (by stratifying the data on herd and type of cultured organism), the OR and 95% CI for a clinical mastitis case to occur in lactation number 2, 3, 4, 5, 6, 7 and 8 relative to the first lactation were 1.65 (1.32–2.06), 2.35 (1.88–2.94), 2.51 (1.96–3.23), 2.79 (2.10–3.70), 2.38 (1.61–3.49), 2.13 (1.24–3.65) and 1.85 (0.71–4.85), respectively ( $\chi^2 = 72.41$ ,  $df = 7$ ,  $p < 0.0001$ ).

The proportional prevalences of cultured microorganisms were calculated for quarter samples of 978 cows affected by clinical mastitis and are shown in Table 1. The most prevalent microorganisms isolated were *Escherichia coli* (51.2%), *S. dysgalactiae* (10.7%), CNS (7.7%), *Sta. aureus* (6.4%) and *A. pyogenes* (4.9%).

Since cows could have multiple infections in one quarter and/or multiple quarters infected, the proportional prevalences of microorganisms were also calculated for cow cases. Most clinical-mastitis cases were associated with coliform bacteria (60.2% of cases), environmental streptococci (18.6%), coagulase negative staphylococci (8.7%) and samples from which no bacterial growth was detected (8.1%) (Fig. 2).

A total of 1190 quarters was affected with clinical mastitis in 1089 cows (unknown quarter in 36 cases). A single quarter was affected in 92.6% (1008/1089) of the cows, 2 quarters in 6.4% (70/1089), 3 quarters in 0.2% (2/1089) and 4 quarters in 0.8% (9/1089) of the affected cows. The distribution of the affected udder quarters was

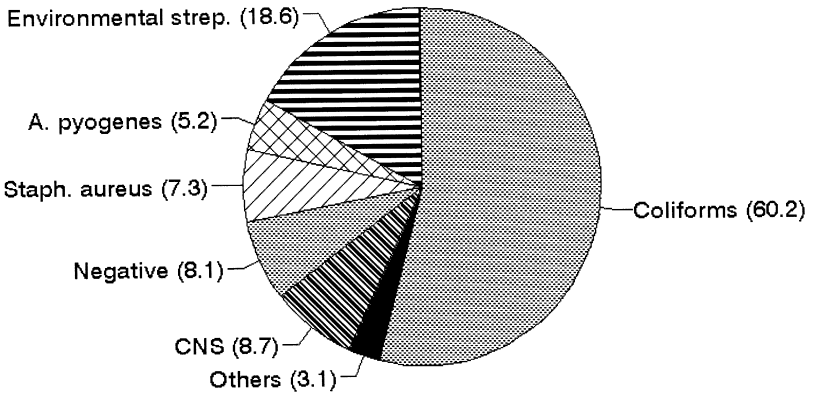


Fig. 2. Prevalence of cultured microorganisms (% of cow-cases) in milk samples from 978 cases of clinical mastitis in Holstein dairy cows in seven Israeli herds in 1989–1993.

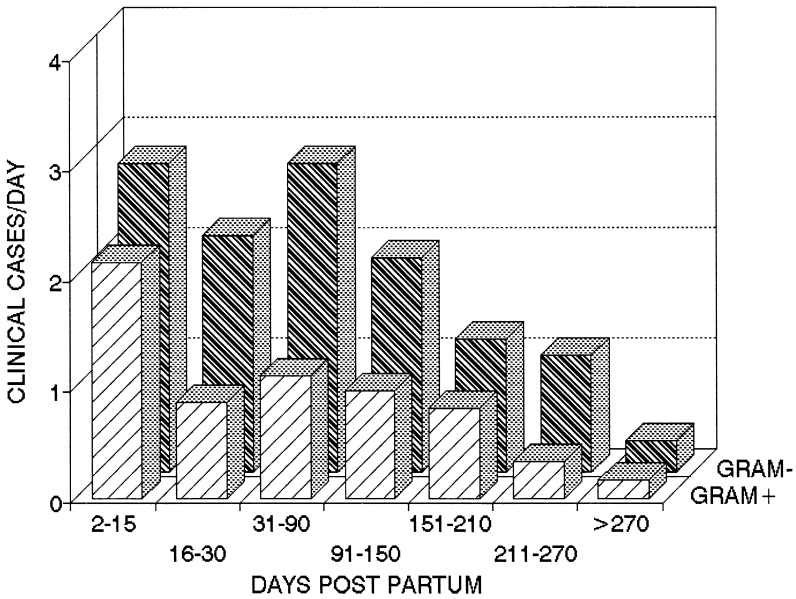


Fig. 3. Distribution of days from calving at diagnosis of 1124 cases of clinical mastitis in Holstein dairy cows in seven Israeli herds in 1989–1993 affected by Gram-positive or Gram-negative organisms.

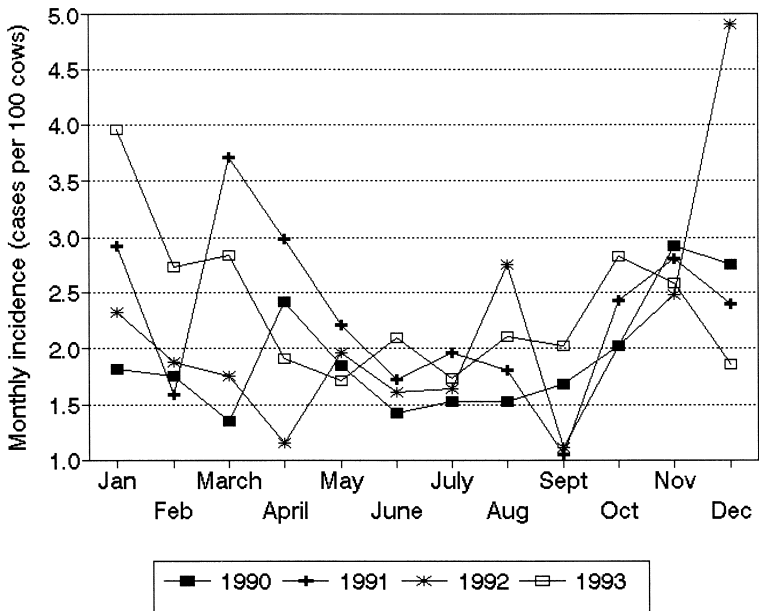


Fig. 4. Monthly incidence of 1124 cases of clinical mastitis in Holstein dairy cows in seven Israeli herds in 1990–1993.

33.5% right rear, 31.2% left rear, 18.2% right front and 17.1% left front quarter. No association could be demonstrated between type of cultured microorganism, defined as environmental streptococci, coliforms and all others, and udder quarter location ( $\chi^2 = 4.18$ ,  $df = 6$ ,  $p = 0.65$ ). Similarly, no association was found between parity and clinical mastitis quarter location ( $\chi^2 = 23.91$ ,  $df = 21$ ,  $p = 0.30$ ).

Most cases of clinical mastitis occurred in the early stages of lactation (Fig. 3), with 51.4% of all cases, 52.3% of coliform cases and 54.6% of environmental streptococci mastitis cases occurring during the first 4 months of lactation. The median number of days in milk at diagnosis was 117.5 days.

The monthly incidence risk of clinical mastitis in the years 1990–1993 is presented in Fig. 4. Despite the marked variability among years, there was a significant difference in seasonal incidence with a lower incidence in the summer months. Using Edwards' test for a simple harmonic curve (with adjustment for population size), the ratio of peak to low incidence was 1.62 with a calculated peak incidence in January ( $\chi^2 = 29.36$ ,  $df = 2$ ,  $p < 0.0001$ ).

The proportions of the outcome categories for all cases were 83.5% total recovery, 5.8% blind quarter and 10.8% of all cases were culled.

#### 4. Discussion

Unknown or poor case definition combined with reporting or submission bias are common problems affecting field studies. It was suggested by Bartlett et al., 1986 that these problems "can best be eliminated by having an investigator live on each of the study farm and observe every animal each day." The design and conduct of this field study enabled us to minimize such inaccuracies. The present analysis included only acute, severe clinical mastitis cases as defined; milder and more chronic cases, although examined by the attending veterinarian, were not included. The incidence risks and lactational risks we found are very similar to those previously reported (Bartlett et al., 1986, 1992; Erskine et al., 1988; Gröhn et al., 1990). The increased risk of clinical mastitis with parity (Gröhn et al., 1990), the quarter distribution (Gonzalez et al., 1990) and marked seasonal incidence (Erskine et al., 1988; Gonzalez et al., 1990) conform with previous reports. The marked seasonal incidence seems to link clinical mastitis with the colder and rainy winter season. The median number of days in milk at diagnosis found in this study was considerably longer than those previously reported (Gröhn et al., 1990). This difference should most probably be attributed to the fact that only the first diagnosis of mastitis in each lactation was considered. However, this might also be due to selection or reporting bias which occurred in previous studies where farmers tended to be more concerned with post-partum cows while those in advanced lactation were overlooked. The extremely high proportion of coliform mastitis, 60.2% of cases, is probably unprecedented. This proportion might actually be even higher considering the possibility that freezing of milk samples may reduce the number of coliform isolates and increase the number of CNS isolates (Schukken et al., 1989). Furthermore, it was suggested that most negative samples (8.1% in this study) could in fact be coliforms



(Erskine et al., 1988; Gonzalez et al., 1990; Smith, 1983). Similar high proportion of coliforms associated clinical mastitis was previously reported by us in clinical field trials (Shpigel et al., 1994, 1996).

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