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Long term effects of *Escherichia coli* mastitis

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ABSTRACT

Escherichia coli is one of the most frequently diagnosed causes of bovine mastitis, and is typically associated with acute, clinical mastitis. The objective of the present study was to evaluate the long term effects of intramammary infections by *E. coli* on milk yield and quality, especially milk coagulation.

Twenty-four Israeli Holstein cows diagnosed with clinical mastitis due to intramammary infection by *E. coli* were used in this study. Mean lactation number, days in milk (DIM) and daily milk yield (DMY) at the time of infection was 3.3 ± 1.3, 131.7 days ± 78.6 and 45.7 L ± 8.4, respectively. DMY, milk constituents, somatic cells count (SCC), differential leukocytes count and coagulation parameters were subsequently assessed.

Two patterns of inflammation were identified: 'short inflammation', characterized by <15% decrease in DMY and <30 days until return to normal ($n = 5$), and 'long inflammation', characterized by >15% decrease in DMY and >30 days to reach a new maximum DMY ($n = 19$). The estimated mean loss of marketable milk during the study was 200 L/cow for 'short inflammation' cases, and 1500 L/cow for 'long inflammation' ones. Significant differences between 'short' and 'long inflammation' effects were found in almost all parameters studied. Long-term detrimental effects on milk quality were found regardless of clinical or bacteriological cure of affected glands.

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Introduction

Bovine mastitis is a disease of high economic importance. Economic losses at the farm level are mainly due to treatment costs, loss of milk yield, lower milk value due to high somatic cell count (SCC) and increased risk of culling (Seegers et al., 2003; Huijps et al., 2008; Hogeveen et al., 2011). In addition, recent studies have shown that economic losses due to mastitis reach beyond farm level, as milk from glands with mastitis increases bulk SCC and induces changes in milk properties that affect production of dairy products (Leitner et al., 2006, 2011; Merin et al., 2008; Le Maréchal et al., 2011).

Escherichia coli, one of the most frequent causes of bovine mastitis, is typically associated with clinical, acute mastitis. Nonetheless, clinical symptoms vary widely from mild to per-acute, and persistent infections have been also reported (Dopfer et al., 1998; Bradley, 2002). Treatment by antimicrobials or quarter drying-off might eventually clear the bacterial infection, but full recovery of the gland may take a longer time than clinical recovery. Thus, the economic impact of *E. coli* mastitis should include post-infection effects on milk quality and not just milk yield loss.

The objective of the present work was to study the long term effects of *E. coli* intramammary infections with diverse clinical presentations on milk yield and milk quality, especially on milk coagulation parameters.

Materials and methods

Animals

Twenty-four Israeli Holstein cows in a commercial dairy herd, which had been diagnosed with clinical mastitis due to intramammary infection by *E. coli*, were used in this study. The herd included a total of 220 cows producing about 11,400 L of milk per 305 day lactation and had an average bulk tank SCC of 200,000 cells/mL. Cows were milked three times per day (at 05:00, 13:00 and 20:00 h) and were fed a typical Israeli total mixed ration containing 65% concentrate and 35% forage (17% protein). Food was offered ad libitum in mangers located in sheds.

Cow selection

Cows identified by farm staff as having clinical mastitis had a milk sample taken aseptically by the farm staff. This was then stored at –20 °C. Within 8 h, an additional sample was taken by the researchers, and both samples were transferred to the laboratory. Cows were included in the study only when *E. coli* was isolated from both samples. After the second sampling, all animals were treated once daily for 3 days with 30 mL of procaine penicillin (PEN30, ABIC Biological Laboratories), 30 mL gentamycin (Gentaject, ABIC Biological Laboratories) and 20 mL of flunixin meglumine (Finadyne, MSD Animal Health) according to the usual treatment protocol in the study herd. Drying-off treatment was performed with intramammary casein hydrolyzate (Leitner et al., 2007).

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For bacteriological examination, 10 μL of each milk sample were inoculated onto blood agar (5% washed sheep red blood cells) and MacConkey agar plates (Bacto-Agar, Becton Dickinson). Plates were incubated at 37 °C and examined for bacterial growth after 18 and 42 h.

Data collected from the herd book for the selected cows included: number of lactations; days in milk (DIM); days pregnant (DIP); daily milk yield (DMY) (L/day); % milk fat, protein and lactose; SCC; and previous disease. Subsequently milk yield and conductivity data were collected daily (Afimilk, Kibbutz Afikim, Israel). Aseptic milk samples were collected daily during the first week, and then weekly from infected glands for bacteriological and SCC examination (Coulter cell counter – Z1, Coulter Electronics). In addition, 300 mL of milk were collected and used for evaluation of milk gross composition (protein, casein, fat, lactose and urea contents) and SCC using Milkoscan 6000 and Fossomatic 360 (Foss Electric), respectively.

To evaluate the status of the mammary gland immune reaction to infection, somatic cell differentiation was performed using flow cytometry (FACs Calibur flow cytometer, Becton-Dickinson) using anti-bovine monoclonal antibodies (VMRD) (Leitner et al., 2003). Monoclonal antibodies used were: anti-CD18/11a – BAT 75A (immunoglobulin [IgG-1]); anti-CD4 – GC 50A1 138A (IgM); anti-CD8 – CACT 80C (IgG-1); anti-CD21 – BAQ 15A (IgM); anti-CD14 – CAM 36A (IgG-1); and anti-polymorphonuclear (PMN) (G1) (IgM). All monoclonal antibodies used were species-reactive with bovine cells. Secondary polyclonal antibodies (Caltag Laboratories) used were goat anti-mouse IgG-1 conjugated with Tri-Color (TC) and goat anti-mouse IgM conjugated with FITC.

Milk coagulation time (R) and curd firmness (CF) were tested using Optigraph (Ysebaert) on the 300 mL milk samples described earlier. The whole procedure continued until the gland completely recovered or became highly altered. Parameters used to indicate gland recovery included milk yield, SCC, %CD18+ and CF, which were compared to expected normal values. Glands considered highly altered were those from which milk secretion almost ceased due to the inflammatory process, or milk yield was too low with too high a SCC, and were therefore dried-off.

Loss of marketable milk during the study was determined based on: (1) quantity of milk discarded due to antibiotic treatment (daily milk yield before infection × 6 days); (2) milk lost due to decreased milk yield (area between the predicted lactation curve and the actual one); (3) quantity of milk discarded due to high SCC, assuming that infected quarters were milked into quarter-jars, and not into the bulk milk tank when SCC was $>1.5 \times 10^6$/mL. The predicted lactation curve of a cow was calculated based on a model from the Israeli Herdbook (Israel Cattle Breeders Association, 2012) for a 305-day lactation. For each particular cow, corrections were automatically made to the predicted curve after monthly milk yield measurements. The last prediction before the mastitis event was used for the comparison.

Bacterial identification and antimicrobial susceptibility

Bacteria were identified based on conventional tests, including colony morphology on MacConkey and blood agars, and oxidase, catalase, triple sugar iron, urea and indole tests (Blum et al., 2008). Antimicrobial susceptibility testing was performed by agar disk diffusion in accordance with CLSI guidelines (CLSI, 2008) using commercially available disks – Dispens-O-Disc (Difco) or BBL Sensi-Disc (Becton Dickinson) – which were used as recommended by the manufacturers.

Pulse-field gel electrophoresis (PFGE)

DNA was digested with XbaI and a standard PFGE technique was used (Blum and Leitner, 2013). Cluster analysis was performed by UPGMA in Bionumerics 6.6 (Applied Maths) and DICE similarity coefficient was calculated using 2% tolerance and 2% optimization.

Statistical analysis

Statistical analyses were performed using JMP software (SAS Institute, 2000). Multiple comparisons between each parameter with expected values for normal glands were made using the Tukey-HSD test. Expected values for normal glands were based on values obtained for non-infected, early to mid-lactation glands from previous studies (Leitner et al., 2011, 2012).

Results

Mean lactation, DIM and DMY at the time of infection of the 24 cows were 3.3 ± 1.3, 131.7 ± 78.6 days and 45.7 ± 8.4 L, respectively. Two patterns of inflammation were identified: 'short inflammation', consisting of cows that returned to maximum DMY within 30 days and in which maximum DMY decrease after mastitis was <15% compared to DMY before mastitis; and 'long inflammation', consisting of cows that reached a new maximum DMY in >30 days and in which decrease in post mastitis maximum DMY was >15% in comparison to DMY before mastitis. Tables 1 and 2 summarize the mean and SE of milk and cellular parameters evaluated. The mean for over days from mastitis detection was calculated for all the cows/glands which continued to produce milk, regardless of whether bacteria were isolated or not.

Five cows were classified as having a 'short inflammation' pattern. These cows completely recovered within 20–30 days. In this period of time, no bacteria were further detected in milk, DMY returned almost to the level before mastitis detection, milk coagulation parameters returned to expected values and SCC and leukocyte distribution returned to normal (Tables 1 and 2) (Leitner et al., 2011, 2012). One representative cow (3013) that completely recovered from 'short inflammation' is presented in Fig. 1a. Milk loss related to the period of antibiotic treatment and milk withhold time (3 + 3 days, respectively) was 39 L/day × 6 = 234 L. However, it took about 15–25 days for milk to return to acceptable quality levels. Since such milk is not supposed to be added into the bulk tank, and should therefore be discarded (9.7 L/day × 20 days = 194 L), estimated overall milk loss for this cow was actually about 430 L.

Table 1
Mean and SE of milk yield, log somatic cell count (SCC), fat, protein, lactose, coagulation time (R) and curd firmness (CF) of glands showing 'short' or 'long inflammation' patterns due to *E. coli* intramammary infection.

	Days[a]	Bac+/cows[b]	Milk (L/day)	SCC (Log)	Fat (g/L)	Protein (g/L)	Lactose (g/L)	R (s)	CF (V)
Uninfected[c]			45	4.69	39	35	50	1000	14
Short ($n = 5$)	1	5/5	12.5 ± 5*	6.80 ± 0.22*					
	4	0/5	39.4 ± 4	6.54 ± 0.19*	27.4 ± 2*	38.3 ± 2	42.5 ± 2*	3014 ± 320*	3.4 ± 1.2*
	12	0/5	43.2 ± 2	5.81 ± 0.65*	45.8 ± 10	47.6 ± 6	43.1 ± 2*	2445 ± 1135	8.4 ± 2.1
	20	0/5	44.7 ± 2	5.04 ± 0.48	37.9 ± 3	38.6 ± 2	50.0 ± 2	1319 ± 416	11.4 ± 0.1
	28	0/5	43.9 ± 2	5.00 ± 0.61	37.2 ± 3	38.9 ± 2	49.4 ± 2	1314 ± 467	12.1 ± 0.2
Long ($n = 19$)	1	19/19	11.2 ± 2*	7.01 ± 0.15*					
	4	4/19	13.6 ± 3*	6.74 ± 0.34*	26.6 ± 3*	39.0 ± 3	35.5 ± 0*	>5000*	0*
	12	3/19	14.1 ± 4*	6.50 ± 0.65*	24.1 ± 3*	40.4 ± 2	43.6 ± 1*	4137 ± 76*	1.2 ± 0.4*
	20	3/17	17.4 ± 3*	6.31 ± 0.54*	23.4 ± 4*	35.8 ± 1	42.9 ± 0*	2655 ± 361*	5.6 ± 1.0*
	28	1/15	32.6 ± 5*	6.41 ± 0.68*	19.6 ± 1*	34.1 ± 2	45.3 ± 0*	3152 ± 392*	6.1 ± 0.7*
	35	1/13	37.3 ± 3	6.19 ± 0.29*	22.9 ± 3*	34.9 ± 1	43.6 ± 3*	2621 ± 454*	7.1 ± 0.8*
	49	1/10	37.6 ± 4	6.60 ± 0.47*	17.4 ± 3*	35.2 ± 1	42.4 ± 3*	3503 ± 611*	1.9 ± 0.8*
	63	1/7	34.3 ± 6	6.52 ± 0.54*	22.2 ± 4*	32.9 ± 1*	45.1 ± 3*	>5000*	0*
	135	1/2	35.6 ± 4	6.75 ± 0.27*	22.8 ± 4*	33.6 ± 1*	43.8 ± 4*	4277 ± 319*	1.6 ± 1.2*

*Different from values of uninfected cows, $P < 0.001$.
[a] Days from mastitis detection.
[b] Bacteria (*E. coli*) positive cows over number of cows tested at that day.
[c] Expected values of uninfected glands (Leitner et al., 2011).

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