

## Relationship Between Subclinical Mastitis And Hygiene Scores In Dairy Cows During Transition Period

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### ABSTRACT

The objective of this study was to investigate the correlation among udder, leg hygiene scores, and subclinical mastitis (SM), somatic cell count (SCC), especially during transition period in different seasons. Individual cow SCC data are summarized for all milking cows (No. = 2412) between October 2006 and December 2007. Udder and leg hygiene scores were assessed through bimonthly schedule visiting during two periods from April to June and from October to December using four point scales ranging from one (clean) to four (very dirty). The prevalence and new infection rates (NIR) of SM were 9.4% and 5.8% respectively. The highest rate of infection and the highest proportions of 3 and 4 udder hygiene scores (UHS), lower hind leg hygiene scores (LHS) and upper hind leg and flank hygiene scores (ULHS) were during the early lactating stages. Dry cow new infection and cure rates were 89.5% and 50.0 % respectively, whereas 36.7 % of cows that had SCC  $\geq$ 200,000 cell/ml during the dry period were culled. There was a significant negative correlation between milk yield and SCC. The rate of SM and somatic cell scores (SCS) were higher during April to June than those during October to December especially in uniparus cows. Hygiene scores for the udder were significantly correlated with SCS. Moreover animals with udders categorized as dirty were 2.8 times more likely to have SM compared with those categorized as clean. It could be concluded that, UHS should be routinely performed as a quality control measure. In addition, more attention should go to post-fresh hygiene especially during hot seasons.

### INTRODUCTION

Mastitis is one of the most economic diseases affecting dairy cows throughout the world. Somatic cell counts (SCC) has been identified as an accurate indirect method to predict mastitis and as an indicator of milk quality and health of cows (1). A cow has subclinical mastitis (SM) when the SCC  $\geq$ 200,000 cell/mL (2). It is important on farms to know that may exceed a cut off value what is needed to bring it below the bonus program requirements, because there is a negative relationship between SCC and milk production, casein composition and shelf life of processed fluid milk (3).

Moreover intramammary infections (IMI) around parturition, either clinical or subclinical, will lead to considerable financial losses and have negative effects on reproductive performance (4). Only a limited number of studies have looked at this problem, leaving many questions on how to prevent IMI pre- and postpartum especially in heifers (5-7).

The incidence of IMI and bacterial numbers in milk was correlated with the number of mastitis pathogens present on the teat end (8). The environment and the cows themselves were cleaner for herds that produced milk with lower SCC values compared with those having higher bulk tank SCC values (9). Hygiene scoring systems have been used to assess the cleanliness of cows and the farm environment (9-11). But there is still little knowledge about the relationship between individual cow hygiene scores and SM. The objective of this study was to investigate the correlation among udder, leg hygiene scores and SCC especially during pre- and postpartum period, as well as to assess the rate of SM and new infection rate (NIR) during different seasons.

### MATERIAL AND METHODS

#### Animals

A total 2412 uniparus and multiparus Holstein cows' dataset were collected between

October 2006 and December 2007. The animals were fed on Machimora farm in Hokkaido, Japan. The number of lactating cows per month herd ranged from 151- 170 cows. All animals were housed in free stalls and were milked twice daily. This farm was enrolled in an official dairy herd improvement association program (DHIA), has SCC less than 200,000 cells/ml.

### Data Collection

The individual cow SCC was determined monthly by using a Fossomatic cell counter (Foss Electric, Hillerød, Denmark). SCC test-day as well as somatic cell scores (SCS) records between 6 and 305 days in milk (DIM) were available for inclusion in the analysis. Lactation average SCS was defined as the mean of all test day records within lactation. Each lactation was further divided into 3 stages: early lactation (6 to 60 DIM), mid lactation (61 to 120 DIM), and late lactation (121 to 305 DIM). Average SCS within these stages was calculated separately for each lactation stage.

### SM and NIR Estimates

The prevalence of SM was determined from the number of cows that had SCC  $\geq 200,000$  cells/ml on any test days divided by the total number of lactating cows tested during the studying period. New infection was defined as: 1) those cows that had an SCC  $\geq 200,000$  cells/ml on the first test after calving or 2) those with SCC  $< 200,000$  cells/ml in the previous month, but  $>200,000$  cells/ml in the present month (12).

### Udder and leg hygiene scores

The herd was visited on a bimonthly schedule during two periods from April to June and from October to December. Hygiene scores were based on a system devised previously (13). Udder and lower legs of studied animals were compared to model animals depicted in photos on the scoring sheet and given a score 1–4 scale on three regions of their bodies: lower hind leg; udder; flank and upper hind leg. Lower scores were indicative of cleaner body regions. A score 1 indicate little flecks or no manure in the region. A score 2 meant there was minor splashing of

manure in the region. A score 3 indicated distinct plaques of manure with some hair visible, and a score of 4 denoted confluent plaques of manure covering the area.

### Statistical Analysis

The scoring data and the SCC are processed to present odds ratios (OD) (14). Moreover the obtained data were compared and analyzed by Scheffe's multiple comparison F test and Chi Square using SAS (15).

## RESULTS

Out of 2412, 227 dairy cows had subclinical mastitis with a rate of 9.4% and the NIR was 5.8% (139/2,412). Cows in early lactating stage were more affected (9.9%, 38/385) than those in mid (4.1%, 18/443) and late stages (5.2%, 83/1,584) (Table 1). The result revealed also that 89.5% (34/38) of the new infection in the early stage was occurred during dry period. Out of 30 cows that had SCC  $\geq 200,000$  cell/ml and entered dry period 13.3% (4) were failed to be treated and passed infected in the following lactating stage, 50.0 % (15) were treated and 36.7 % (11) were culled (Table 2). There was a significant negative correlation between SCC and milk yield ( $r = 0.704$ ,  $P < 0.01$ ) (Fig 1).

The rates of SM and the proportion rates of 3 and 4 hygiene scores (UHS, LHS and ULHS) of the post-fresh cows were higher than those of the other lactating stages. The proportion rates of SM and SCS were higher during April to June than those during October to December. Dairy heifer had higher 3 and 4 UHS %, SM and SCS during April to June than during October to December (Table 3).

Animals with udders categorized as dirty were 2.8 times more likely to have SM compared with those categorized as clean. Cows with UHS, LHS or combined udder and leg hygiene scores categorized as dirty were more likely to have SCC  $\geq 200,000$  cell/ml compared with those categorized as clean. Moreover hygiene scores for the udder were significantly correlated with SCS (Table 4).

**Table 1.** Prevalence and new infection rates of subclinical mastitis during the stage and season of lactation

Lactation stage	1 <sup>st</sup> Lactating season		2 <sup>nd</sup> Lactating season		3 <sup>rd</sup> Lactating season		≥ 4 <sup>th</sup> Lactating season		Total	
	SCC (cell/mL)	NIR (%)	SCC (cell/mL)	NIR (%)	SCC (cell/mL)	NIR (%)	SCC (cell/mL)	NIR (%)	SCC (cell/mL)	NIR (%)
	≥200,000 (%)		≥200,000 (%)		≥200,000 (%)		≥200,000 (%)		≥200,000 (%)	
Early	8.7 (13/149)	7.4 (11/149)	7.4 (6/81)	6.2 (5/81)	18.0 (9/50)	14.0 (7/50)	15.2 (16/105)	14.3 (15/105)	11.4 (44/385)	9.9 (38/385)
Mid	4.8 (8/167)	2.4 (4/167)	11.1 (11/99)	6.1 (6/99)	8.5 (6/71)	7.0 (5/71)	4.7 (5/106)	2.8 (3/106)	6.8 (30/443)	4.1 (18/443)
late	4.2 (26/626)	2.9 (18/626)	14.4 (43/298)	6.4 (19/298)	13.9 (47/339)	7.7 (26/339)	11.3 (37/321)	6.2 (20/321)	9.7 (153/1,584)	5.2 (83/1,584)
Total	4.2 (47/942)	3.5 (33/942)	12.6 (60/478)	6.3 (30/478)	13.5 (62/460)	8.3 (38/460)	10.9 (58/532)	7.1 (38/532)	9.4 (227/2,412)	5.8 (139/2,412)

NIR: new infection rate. Numbers in parenthesis indicate number of animals/total number.

Table 2. Dry period length, new infection, cure and culling data.

	%
Early new infection (n= 38)	
Infection during dry period	89.5 (34/38)
Cow enter dry period with SCC >200,000 (n= 30)	
Dry cow cure rate	50.0 (15/30)
Dry cow failed treatment (50.0%, 15/30)	13.3 (4/30)
Passed infected	36.7 (11/30)
Culled cow	

Numbers in parenthesis indicate number of animals/total number.

Table 3. Hygiene scores (udder and leg), somatic cell scores and rate of subclinical mastitis in different lactation stages during April to June and October to December.

Lactation stage	Period	Hygiene Scores												Somatic cell scores (SCS)				Prevalence <sup>a</sup> rate of SM		New Infection %			
		Udder hygiene scores				Lower leg hygiene scores				Upper leg & Flank hygiene scores				SCS (Mean ±SD)		0-3 SCS %		7-9 SCS %		Heifer	Cow	Heifer	Cow
		Heifer		Cow		Heifer		Cow		Heifer		Cow		Heifer	Cow	Heifer	Cow						
Mean ±SD	%Hygiene score 3&4	Mean ±SD	%Hygiene score 3&4	Mean ±SD	%Hygiene score 3&4	Mean ±SD	%Hygiene score 3&4	Mean ±SD	%Hygiene score 3&4	Mean ±SD	%Hygiene score 3&4	Mean ±SD	%Hygiene score 3&4	Heifer	Cow	Heifer	Cow	Heifer	Cow	Heifer	Cow		
Post-fresh	April to June	2.9 ±0.8	71.4 (30/42)	3.3 ±0.8	81.6 (31/38)	3.6 ±0.5	97.6 (41/42)	3.6 ±0.6	97.4 (37/38)	3.3 ±0.8	83.3 (35/42)	3.2 ±0.7	81.6 (31/38)	3.0 ±1.5*	2.2 ±1.7*	66.7	78.9	4.5	5.1	21.4	21.1	14.3	15.8
	October to December	2.9 ±0.8	65.3 (32/49)	3.3 ±0.7	81.5 (66/81)	3.9 ±0.4	100 (49/49)	3.7 ±0.6	93.8 (76/81)	3.3 ±0.7	83.7 (41/49)	3.3 ±0.7	86.4 (70/81)	1.9 ±1.5	1.8 ±1.8	96	91.4	4.0	3.0	6.7	12.8	6.7	8.5
	Total	2.9 ±0.8*	68.1 (62/91)	3.3 ±0.8*	81.5 (97/119)	3.8 ±0.5*	98.9 (90/91)	3.7 ±0.6*	95.0 (113/119)	3.3 ±0.8*	82.4 (75/91)	3.2 ±0.7*	84.9 (101/119)	2.4 ±1.6	1.9 ±1.8	82.4	87.7	3.2	3.2	10.3	16.5	10.3	11.8
Late Lact	April to June	3.0 ±0.9	64.0 (16/25)	2.9 ±0.7	66.7 (18/27)	3.2 ±0.7	84.0 (21/25)	3.3 ±0.6	92.6 (25/27)	3.0 ±0.8	68.0 (17/25)	2.9 ±0.8	63.0 (17/27)	2.2 ±1.4*	3.8 ±1.1*	88.0	66.7	4.0	0.0	4.4	10.8	4.4	5.7
	October to December	2.7 ±0.8	53.8 (7/13)	2.8 ±0.8	60.0 (12/20)	3.3 ±0.8	84.6 (11/13)	3.2 ±0.7	85.0 (17/20)	2.9 ±0.8	69.2 (9/13)	3.0 ±0.9	65.0 (13/20)	1.8 ±0.9	3.3 ±1.3	92.3	75.0	0.0	0.0	4.0	10.6	4.0	4.5
	Total	2.9 ±0.9*	60.5 (23/38)	2.9 ±0.8*	63.8 (30/47)	3.2 ±0.7*	84.2 (32/38)	3.2 ±0.6*	89.4 (42/47)	3.0 ±0.8*	68.4 (26/38)	2.9 ±0.8*	63.8 (30/47)	2.0 ±1.4	3.0 ±1.2	89.5	70.2	2.6	0.0	4.2	10.7	4.2	5.1
Far off	April to June	1.9 ±0.6	14.3 (3/21)	2.4 ±0.8	37.3 (28/75)	3.3 ±0.7	85.7 (18/21)	3.4 ±0.7	85.3 (64/75)	2.0 ±0.7	19.0 (4/21)	2.6 ±0.9	56.0 (42/75)										
	October to December	2.4 ±0.6	39.0 (16/41)	2.2 ±0.7	27.2 (31/114)	3.4 ±0.7	85.4 (35/41)	3.6 ±0.6	92.1 (105/114)	2.7 ±0.7	53.7 (22/41)	2.6 ±0.8	50.9 (58/114)										
	Total	1.3 ±0.7	30.6 (19/62)	2.3 ±0.8*	31.2 (59/189)	3.4 ±0.7*	85.5 (53/62)	3.5 ±0.7*	89.4 (169/189)	2.5 ±0.8*	41.9 (26/62)	2.6 ±0.8*	52.9 (100/189)										
Close up	April to June	1.8 ±0.6	13.3 (2/15)	1.9 ±0.9	27.6 (8/29)	2.7 ±0.8	53.3* (8/15)	2.6 ±0.7	48.3 (14/29)	2.4 ±0.7	46.7 (7/15)	2.8 ±0.9	62.1 (18/29)										
	October to December	2.1 ±0.5	21.4 (3/14)	2.2 ±0.7	25.0 (10/40)	3.2 ±0.8	78.6 (11/14)	3.2 ±0.9	75.0 (30/40)	2.4 ±0.6	42.9 (6/14)	2.5 ±0.8	45.0 (18/40)										
	Total	2.0 ±0.6*	17.2 (5/29)	2.1 ±0.8*	26.1 (18/69)	3.0 ±0.8*	65.5 (19/29)	2.9 ±0.9*	59.4 (44/69)	2.4 ±0.6*	44.8 (13/29)	2.6 ±0.8*	52.2 (36/69)										

Numbers in parenthesis indicate number of animals/total number. # Prevalence rate of subclinical mastitis (SM) mean percent of cows that had SCC > 200,000 cell/mL. Means in the same column having different superscripts were significantly different (P > 0.05). \* Significant when compared in the same column within the same stage (\*P < 0.05; \*\*P < 0.01).

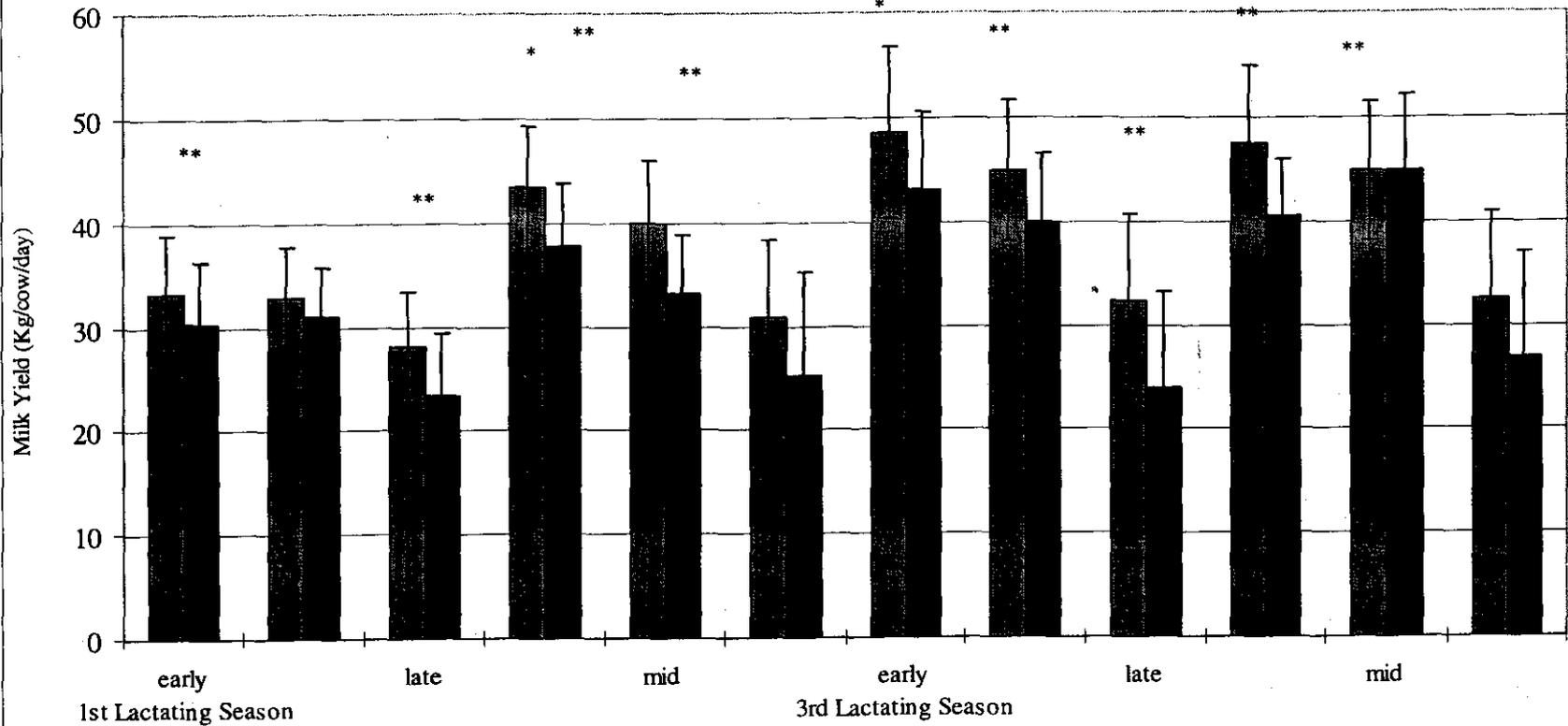
Table 4. Relation between hygiene scores (udder, lower leg and upper leg &amp; flank) and somatic cell counts (SCC)

	Hygiene scores		
	clean <sup>1</sup>	dirty <sup>2</sup>	Total
Udder hygiene scores			
< 200,000 SCC	78	180	258
≥ 200,000 SCC	5	32	37
Total	83	212	295
Proportion > 200,000 SCC	6.0%	15.1%	12.5%
$\chi^2$		4.5	P = 0.03*
Odds ratio		2.8	
Lower leg hygiene scores			
< 200,000 SCC	16	242	258
≥ 200,000 SCC	2	35	37
Total	18	277	295
Proportion > 200,000 SCC	11.1%	12.6%	12.5%
$\chi^2$		0.04	P = 0.850
Odds ratio		1.2	
Upper leg hygiene scores			
< 200,000 SCC	56	202	258
≥ 200,000 SCC	7	30	37
Total	63	232	295
Proportion > 200,000 SCC	11.1%	12.9%	12.5%
$\chi^2$		0.2	P = 0.698
Odds ratio		1.2	
Combined udder and leg hygiene scores			
< 200,000 SCC	37	221	258
≥ 200,000 SCC	3	34	37
Total	40	255	295
Proportion > 200,000 SCC	7.5%	13.3%	12.5%
$\chi^2$		1.1	P = 0.300
Odds ratio		1.9	

<sup>1</sup>Combined data for scores 1 and 2<sup>2</sup>Combined data for scores 3 and 4.

Fig 1. Relation between milk yield and somatic cell counts (cell/mL) and milk yield (kg/cow/day) according to stage and lactating seasons

( $r = 0.704, P < 0.01$ )



\*\* P<0.01 \* P<0.05

■ Milk yeild normal      ■ Milk yeild SM

## DISCUSSION

Early diagnosis of mastitis is vital because changes in the udder tissue take place much earlier than they become apparent. Measurement of monthly SCC from individual cows is widely used for estimating and monitoring udder infection dynamics on farms (12). Previous studies had suggested that SCC is normally elevated during the first 2 weeks of lactation, followed by a rapid decrease (16). However, more recent studies have demonstrated that cow level SCC declines more rapidly than previously thought and 95% of culture negative quarters have SCC less than 200,000 cell/mL by the fourth milking (10). DHIA rules mandate that only SCC data from cows 6 DIM or more at the first test are stored and the maximum interval between tests in the current study was 30 days. Estimation of NIR using a simple SCC threshold, or a more complex threshold based on a percentage change from previous test value will undoubtedly improve the accurate determination of true IMI and non-infection (12).

The occurrence rate of SM was 14.5% (17). In the present study, the prevalence rate was 9.4% (227/2,412) and the NIR was 5.8% (139/2,412) (Table 1). In contrast, previously studies showed that the prevalence rate of SM was 49.1% in hand-milked and 57.4% in machine-milked cows (18). This variation may be attributed to the prevalence and incidence of subclinical and clinical mastitis depends on factors such as type of housing, management, and environmental factors (19).

The changes of SCC across the dry period can also be utilized to monitor the effectiveness of dry-cow treatment programs. The new infection and cure-rate data may be used to identify herds in which the entire drying procedure requires investigation (12). The results revealed that dry cow new infection and cure rates were 89.5% and 50.0 % respectively, whereas 36.7 % of cows that had SCC  $\geq$ 200,000 cell/ml during the dry period were culled. Similar results reported that 50.5% of new IMI occurred in the nonlactating udder

and the rate of new infection was 5.5 times greater than during lactation (20). There was a significant negative correlation between SCC and milk yield ( $r = 0.7035$ ,  $P < 0.01$ ). Similar results were reported previously (3 & 21).

Dairy heifers had higher UHS, SM and SCS during April to June than those during October to December. Increasing SCC during April to June may be attributed to the hot weather that may cause increasing the number of bacteria in areas where cow's rest, especially if such areas remain wet or damp. However, management practices to prevent the transmission of pathogens may be practiced less frequently and thoroughly during that period. This increases the exposure of cows to more bacteria in addition to thermal stress making them more susceptible to new infections (22 & 23).

Prevalence rates of SM and the proportion rates of 3 and 4 hygiene scores (UHS, LHS and ULHS) of the post-fresh cows were higher than those of the other lactating stages (Table 3). This result reflects the role of hygiene as one of the most important risk factors of bovine mastitis. Concordant results were reported that poor hygiene of the calving area was associated with an increased prevalence of elevated SCC in heifers (6 & 24).

The results also revealed that animals with udders categorized as dirty were 2.8 times more likely to have SM compared with those categorized as clean. Cows with UHS, LHS or Combined udder and leg hygiene scores categorized as dirty were more likely to have SCC  $\geq$ 200,000 cell/mL compared with those categorized as clean. This result coincided with other reports that showed the presence of mastitis pathogens on teat ends has been correlated with the incidence of IMI (8). The level of hygiene has been detected as higher in herds with low bulk tank SCC than in herds with high bulk tank SCC (9, 10 & 25). This may be attributed that the moisture, mud, and manure present in the environment of the cow are the primary sources of pathogens, and hygiene scores of cows provide visible evidence of exposure to these potential sources.

It could be concluded that, UHS should be routinely performed as a quality control measure just as body condition scores are performed to monitor nutritional management. Each cow with an UHS of  $\geq 3$  has an increased risk of mastitis. In addition, more attention should go to post-fresh hygiene especially during hot seasons. Conducting more studies combining bacteriological culture, SCC recording and hygiene scores on a large number of herds are essential in tracking udder health problems in dairy cattle.

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### الملخص العربي

## العلاقة بين التهاب الضرع الغير مرئي ومستويات النظافة في الأبقار أثناء المرحلة الإنتقالية

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قسم طب الحيوان كلية الطب البيطري جامعة قناة السويس مصر  
قسم طب الحيوان بمدرسة الطب البيطري جامعة راكونو جاكوبين باليابان

تم عمل هذه الدراسة لدراسة العلاقة بين معدل نظافة الضرع والأرجل الخلفية وعدد الخلايا الجسدية في اللبن لا سيما خلال المرحلة الإنتقالية ما قبل وبعد الولادة ، وكذلك تقييم معدل التهاب الضرع الغير مرئي خلال المواسم المختلفة. لقد تم فحص لبن عدد ٢٤١٢ بقرة وعد الخلايا الجسدية الخاصة بكل بقرة في الفترة ما بين أكتوبر ٢٠٠٦ وديسمبر ٢٠٠٧. وكذلك تم قياس معدل نظافة الضرع والأرجل الخلفية عن طريق زيارة القطيع مرتين كل شهر خلال فترتين الأولى من ابريل الى يونيو والثانية من أكتوبر إلى ديسمبر وتم تقييم مستوي النظافة إلى التصنيفات الأربعة بحيث يكون المستوى "١" نظيف ومستوى "٤" قذر جدا.

وأوضحت النتائج ان نسبة الإصابة بالتهاب الضرع الغير مرئي كانت ٩,٤ ٪ بينما كانت نسبة الإصابة الحديثة ٥,٨ ٪. وأن نسبة الإصابة خلال الفترة من ابريل الى يونيو أعلى من تلك التي في الفترة من أكتوبر إلى ديسمبر وخاصة في الأبقار التي ولدت لأول مرة وكان هناك ارتباط سلبي معنوي بين إدرار الحليب ، وعدد الخلايا الجسدية في اللبن. وكانت معدل الإصابة والشفاء من التهاب الضرع خلال فترة التجفيف ٨٩,٥ ٪ و ٥٠,٠ ٪ على التوالي، في حين أن ٣٦,٧ ٪ من الأبقار التي عدد الخلايا الجسدية في لبنها  $\leq 200,000$  خلية / مللي تم استبعادها من القطيع.

كما أوضحت النتائج أيضاً أن أعلى معدل للإصابة بالتهاب الضرع كانت في الحيوانات التي مستوى نظافة الضرع والأرجل الخلفية لها ٣ و ٤ خاصة أثناء المراحل المبكرة بعد الولادة. علاوة على أن الحيوانات ذات الضرع التي مستوى نظافتها ٣ و ٤ تكون ٢,٨ مره أكثر عرضة للإصابة بالتهاب الضرع مقارنة بالحيوانات النظيفة.

ومن هذه الدراسة أتضح لنا أنه ينبغي عمل مراقبة روتينية لمستوى النظافة للضرع والأرجل الخلفية للحكم على كفاءة القطيع وللتحكم في التهاب الضرع وبالإضافة إلى ذلك يجب الإهتمام بالمراقبة الصحية للحيوانات حديثة الولادة خاصة في فصل الصيف.