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(17.28 + 13.64%) and healthy ones (10.89 + 9.49%) ($P = 0.13$). No alteration in viability rates of PMNLs was also observed in infected quarters with *S. dysgalactiae* (46.83 + 23.07%) compared to healthy controls (38.90 + 18.05%) ($P = 0.29$). Therefore, a tendency toward an increase in annexin-V+/PI+ PMNLs from healthy quarters (19.42 + 12.93%) compared to *S. dysgalactiae* infected quarters (13.87 + 16.25%) ($P = 0.057$) was found.

Conclusions: Thus, PMNLs viability in milk can be affected by inflammatory process against infection and/or modulated by mammary pathogens.

P: 435

Decrease in bovine CD14 positive cells in colostrum is associated with the incidence of mastitis after calving

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Objectives: This study was to investigate whether certain characteristics of cows were associated with increased risk of mastitis after calving. We particularly focused on the possible association between the populations of mononuclear cell in colostrum immediately after calving and the incidence of mastitis within 1 week after calving. In addition, the populations of peripheral blood mononuclear cells after calving were also investigated in order to evaluate the systemic immune condition.

Materials and Methods: Six healthy cows (HC) and 8 cows that developed mastitis within 1 week after calving (MC) were used in this study. Quarter colostrum samples and peripheral blood samples were collected from each cow within 2 h after calving. Milk samples were taken from one of the pre-determined quarter of each cow at 1, 2, 3 and 7 d after calving and were used for California mastitis test (CMT) in order to diagnose mastitis. Using flow cytometry, we determined the expression of specific antigens on the surface of macrophages/monocytes (CD14+), T cells (CD3+, CD4+, CD8+, WC1+) and B cells (CD21+) from mononuclear leukocytes in colostrum and peripheral blood of these cattle. In order to determine the significant difference between HC and MC, an unpaired Student's t-test was used.

Results: Eight out of 14 cows were diagnosed to have subclinical mastitis within 1 week after calving in our study. CMT scores of MC were 2 to 4 when they were diagnosed to have mastitis. Flow cytometric analysis of leukocyte subsets of PBMC showed no difference in the percentages of CD3+, CD4+, CD8+, CD14+, CD21+ or WC1+ cells between HC and MC. In contrast, the percentage of CD14+ cells in the colostrum was significantly ($P = 0.018$) lower in MC ($22.04 \pm 5.29\%$) than in HC ($41.26 \pm 4.59\%$), and the other marker positive cells did not differ between MC and HC. We found lower percentage of CD14+ cells in the colostrum of MC, which developed the mastitis within 1 week after calving compared with HC.

Conclusions: Our study indicated the possible association between the percentage of CD14+ cells in the colostrum immediately after calving and the incidence of mastitis during the postpartum period. It is possible that low percentage of bovine CD14+ cells in colostrum is the indicator to predict mastitis after calving. The analysis of the mononuclear leukocyte populations in colostrum may be useful in understanding the immune condition of dairy cows regarding mastitis.

P: 436

Mastitis in Finland – changes during the recent decades

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Objectives: In the Finnish dairy industry great structural changes have occurred during the recent decades. The number of dairy cows per herd and the average milk yield have significantly increased, while the actual number of cows has declined. The number of tie-stall barns has decreased and at the moment, over 60 % of the Finnish dairy cows are kept in free-stall barns. Over 12 % of the cows are milked by automatic milking systems. The aim

of this study was to determine the prevalence of mastitis in Finland in 1991, 2001 and 2010. Somatic cell count (SCC) data from the Finnish herds with production and health recording were used. Factors affecting SCC and its trends were also studied. This kind of wide scale national mastitis survey has not been done before in Finland.

Materials and Methods: The survey was conducted by analyzing data from the national production and health record from the years 1991, 2001 ($n=337\ 314$) and 2010 ($n=272\ 749$). The database includes approximately 80 % of the dairy cows in Finland. SCC was reported for each cow from the first test milk sample of the year. Cows with milk SCC of $\geq 200\ 000$ cells/ml were defined as mastitic. Cows with SCC $\geq 200\ 000$ cells/ml in three or four test milkings of the year, were defined chronically infected. SCC results were also counted separately according to the number of calvings, type of the barn, herd size, average milk yield, area codes, breed of the cow and organic versus normal production.

Results: Prevalence of mastitis had decreased in Finland during the past decades. In 2010 the prevalence was 19.0% and in 2001 20.1%, with statistically significant difference. According to the preliminary calculations factors increasing milk SCC were automatic milking, free-stall barn, organic production, herd size over 60 cows or more, milk yield below Finnish average, number of calvings (three or more) and Holstein-Friesian breed. The prevalence of cows with chronic mastitis was 16.1%, with a 0.6% increase from the year 2001 (15.5%). Data from the year 1991 is still under analysis.

Conclusions: It can be concluded that this survey produced valid long-term information on the changes in mastitis situation in the Finland. In countries where production and health record databases are available, this kind of surveys could be carried out at regular intervals to detect possible trends and factors affecting them. The possible reasons for the changes seen in Finland during the recent decades will be discussed.

P: 437

Microorganisms isolated from milk healthy, clinical or subclinical mastitis goats

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Objectives: The objective of this research was to identify different microorganisms present in milk from healthy, clinical or subclinical mastitis goats.

Materials and Methods: 56 milk samples were obtained of goats from San Luis Potosí and 55 from Coahuila. Bacteriological cultures and isolations were made and identified by different biochemical tests and API identification Microsystems. To know the susceptibility to antibiotics, the Kirby Bauer technique was used.

Results: A total of 95/111 samples (85.58%) presented bacterial growth and eight of them were contaminated, so that, only 87 samples were worked. 100% of samples from Coahuila presented growth, and 71.42% of samples from San Luis Potosí had growth. The most frequently isolated bacteria were: *Staphylococcus chromogenes* 12/87 (13.79%), *Staphylococcus simulans* 9/87 (10.34%), *Staphylococcus xylosum* 9/87 (10.34%), *Staphylococcus lentus* 8/87 (9.19%), *Staphylococcus hominis* 5/87 (5.74%), *Mannheimia haemolytica* 10/87 (11.49%), among others.

Conclusions: The susceptibility to antibiotics was 100% to levofloxacin, trimetoprim sulphametoxazol, and 14.28% were resistant to cephotaxime y cephuroxime.

P: 438

Epidemiological relationship between staphylococcus aureus strains from cows with bovine mastitis and milking environment by pulsed-field electrophoresis

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Objectives: The objective of this research was to investigate the epidemiological link between strains of *S. aureus* isolated from cows with bovine mas-

titis, the sites of location and the routes of transmission.

Materials and Methods: We evaluated 224 strains of *S. aureus* from the milk of cows with subclinical mastitis, bulk tank milk and the following locations in the milking environment, blowers, hoses, vacuum rubber, rubber tank cap balance, hands of milkers and surface of the expansion tank, by the technique of pulsed-field electrophoresis, this technique allows the separation of bacterial isolates in types, or clones, and are associated according to their location, thus verifying their epidemiological link.

Results: The results revealed the presence of 70 different pulsotypes, and of these 150 (67%) were isolated from the milk of cows with subclinical mastitis, 47 (21%) of the blowers and 8 (3.5%) of the hands of milkers. Other places which the isolations were obtained more than one pulsotype were conducting milk hoses, vacuum rubber and rubber tank cap balance. When the *S. aureus* strains were evaluated according to the date of obtaining it was found that no one pulsotype was found more than two consecutive harvests and that there was a wide distribution of pulsotype over the months, and many pulsotypes that which appeared only once in a month. The association between the isolates of *S. aureus* from mastitis cases and from local of isolation are of the extreme epidemiological importance. The milking lines are places of intense management, which may provide conditions for transmission of pathogens to the mammary gland, especially *S. aureus*, if neglected procedures for disinfection of milking equipment and hygiene of the mammary gland during pre-milking.

Conclusions: We conclude that monitoring and a better control of cleaning is required. The sanitizing of milking equipment in addition to the correct cleaning of the hands of milkers it is important to avoid the transmission of the pulsotypes between cows and clonal proliferation in the environment milking.

P: 439

Effectiveness of sodium hypochlorite "in vitro" to strains of *S. Aureus* isolated from the milking environment and from cows with subclinical mastitis

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Objectives: The purpose of this research was to evaluate the effectiveness of sodium hypochlorite "in vitro" to some strains of *S. aureus*, in biofilms and non biofilms, isolated from milking environment and of cows with subclinical mastitis.

Materials and Methods: This study was developed at the University of Minho in Portugal using twelve strains of *S. aureus* derived from milk of cows with subclinical mastitis and of the following local of milking environment, blowers and conductive rubber milk. The techniques used were count of the colonies and the crystal violet method for cells in biofilms and non adherent cells.

Results: The *S. aureus* in biofilms were treated with sodium hypochlorite at concentrations of 150 and 300 ppm in four different times that were five, ten, twenty and thirty minutes. For the cells that were in non biofilms the concentration used was only 150 ppm and this concentration was efficient in eliminate the cells in non biofilms. Unlike, in the biofilm cells at concentrations of 150 ppm of sodium hypochlorite during five minutes of contact was not enough to eliminate biofilm in 33% of staphylococcal strains studied. Already, a concentration of 300 ppm sodium hypochlorite was effective in removing biofilm in four times evaluated. Milking environment hygiene is one of the main points to be monitored and controlled inside a dairy property. The formation of biofilms in the milking equipment origin even more cares because it allows the propagation and maintenance of microorganisms in this environment and in the teats of cows.

Conclusions: We conclude that is important to use other mechanisms to control biofilms due to the damage that the increased concentration of hypochlorite can cause in the blowers and rubbers such as cracks and less time to use these. An alternative is the use of washing equipment with high pressure thus preventing the formation of biofilms in them.

P: 440

Detection and molecular typing of *Streptococcus uberis* isolated from a single farm

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Objectives: *Streptococcus uberis* is a common cause of clinical and subclinical mastitis within dairy herds worldwide. It is found in faecal material and is therefore a common contaminant of dairy farms. It is on this basis that *S. uberis* it is thought to be an environmental pathogen. Despite the advances in the control of other mastitis pathogens, such as *Staphylococcus aureus* and *Streptococcus agalactiae*, mastitis caused by *S. uberis* remains a problem on particular farms. Previous research of pulsed field gel electrophoresis (PFGE) patterns within our laboratory has shown that contagious transmission can occur and particular strains can persist within the environment.

Materials and Methods: In this current study, we received 45 milk samples collected from different cows suffering clinical mastitis from one such farm in New South Wales, Australia. The aim was to isolate and confirm the identity of the pathogen, using a specific PCR and biochemical tests, and to generate a molecular profile of the isolates using PFGE.

Results: *Streptococcus uberis* was isolated from 11 of the 45 samples in pure culture and no significant growth was recorded for the other samples (34). Comparison of the PFGE patterns yielded 8 different molecular profiles amongst the 11 isolates. Importantly, two profiles were isolated from more than one cow.

Conclusions: The discovery of the same molecular profile in multiple milk samples, along with strict contamination controls employed at the farm, would suggest cow to cow transmission. The mechanism by which these strains persist is the subject of our ongoing research.

P: 441

Control of bulk somatic cell counts in dairy farms with an automatic milking system in western France

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Objectives: The dairy cooperative UCAL, located in Vendée (Western France), has been concerned by a quick increase of farms equipped with an automatic milking system (AMS). A degradation of milk quality, mainly the bulk milk somatic cell counts (BMSCC), was observed in almost all farms, without obvious explanations. The aim of this study was to quantify the evolution of BMSCC after the switch to an AMS, and to identify the risk factors associated with this evolution.

Materials and Methods: A survey has been carried out in 49 farms equipped with an AMS during spring 2009. These farms with an average quota of 650 000 Liters have been classified into two groups according to their BMSCC during the first year of use of AMS (correct BMSCC group : farms with less than 4 monthly BMSCC higher than 300 000 cell/mL or farms with 4 monthly BMSCC higher than 300 000 cell/mL but with an improved annual BMSCC compared to the one obtained before AMS; poor BMSCC group : the other farms, with high somatic cells). Risk factors of being in the poor BMSCC group have been identified comparing the management and treatment practices of both groups by multivariable logistic regression. In addition, the BMSCC evolution after the implementation of a systematic disinfection of the liners was described.

Results: On both groups, AMS induced a significant increase in BMSCC for at least 2 years (from 242 000 before to 322 000 cells/mL the 2 years after). The risk factors of being in the poor BMSCC group were : high previous BMSCC, lower culling rate, no priorities for the access of dairy cows to the AMS, higher amount of concentrates distributed in the AMS, low frequency of cleaning of the exercise area, introduction of dried cows in the lactating group before calving, absence of annual foot trimming and late antibiotic treatments. The systematic disinfection of the liners induced on average a significant decrease in BMSCC (from 414 000 before to 305 000 cells/mL the 6 months after).