401 (1455)

BIOLOGICAL CONTROL OF HAEMONCHUS CONTORTUS BY SOME OF THE NEMATOPHAGOUS FUNGI

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Objective: Biological control of gastrointestinal nematodes of ruminants.

Design: Case-control study.

Animals: 50 sheep naturally infected to the ova of Haemonchus contortus

Procedure: A. oligospora (111.37 & 251.83), D. flagrans (583.91) and H. sphaerosporum (381.84) were obtained from CBS of Netherlands and Botany research institute of Iran. Then, nematophagous activity of these isolates was studied after addition of 8000, 20,000 and 100,000 conidia to 1 gram of fecal samples containing 70 ova of Haemonchus contortus per each petri dish. All of the samples were incubated at 25-27°C for 8 days and then, the nematophagous effects of fungal isolates were determined after calculation of third staged larval reduction using Berman method.

Statistical analysis: In order to study of the effects of various numbers of fungal conidia on the reduction of third stage larvae of H. contortus, one-way ANOVA and complementary method of Tukey were used.

Results: Study of nematophagous effects of 8000 conidia of all above-mentioned fungi and 20,000 conidia of A. oligospora (251.82) on the third stage larvae of H. contortus showed that there was not any significant difference as compared with the control groups. But for 20,000 conidia of A. oligospora (111.37) and D. flagrans (583.91) and also 100,000 conidia for all above-mentioned fungi, significant reduction in larvae of H. contortus was observed as compared with the control groups. In H. sphaerosporum, the percentage of larvae reduction for 8000 and 20,000 conidia was determined as 21.46% and 48.99%, respectively. But, the increase of conidia to 100,000 caused only 42.28% reduction in infective larvae so above-mentioned fungus can not function as an effective agent in biological control of H. contortus.

Clinical implications: The present study showed that we can control gastrointestinal nematodes by use of nematode-trapping fungi, A. oligospora CBS 111.37 & CBS 251.82 and D. flagrans CBS 583.91 in suitable conditions along with chemical treatment.

Key words: Nematophagous fungi-Arthrobotrys oligospora-Daddingtonia flagrans-Haptocillium sphaerosporum-Haemonchus contortus

Funding: Garmsar Azad University

402 (1469)

NEMATOPHAGOUS EFFECTS OF NATIVE ISOLATES OF ARTHROBOTRYS OLIGOSPORA ON THE HAEMONCHUS CONTORTUS

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Introduction: One of the problems of controlling the parasitic nematodes is their resistance against anthelmintic drugs. In addition, drug residuals in animal productions including milk and meat also health problems for human has caused to pay attention to biological control programmes to get special position and concern.

Materials and methods: One-hundred and fifty samples of soil (50 samples from Garmsar as a model of tropical and subtropical weather and 100 samples from Mazandaran as a model of moist weather) and 138 samples of faeces were studied for the presence of nematodes-trapping fungi. Three native isolates of Arthrobotrys oligospora were purified based on colony morphology and microscopic characteristics by slide culture method. Then for determining of nematophagous effects of above-mentioned fungi on the third stage larvae of Haemonchus contortus, the different number of fungi conidia as 8000, 20000 and 100000 were added to one gram of donor sheep faeces infected to H. contortus and after 8 days incubation at 27°C number of live larvae were compared with control groups.

Results: Among 100 soil samples tested from Mazandaran, 11 of them harbored A. oligospora of which only three isolate were successfully purified, isolates No.1, 19 & 49. No nematophagous fungi were isolated from soil samples of Garmsar and fecal samples of sheep for both above-mentioned geographical regions. About
nematophagous activity of examined fungi, addition of conidia (8000, 20,000 and 100,000) of these three isolates to faeces containing egg of Haemonchus contortus (EPG = 50) has caused reduction between 55.69% to 96.69% in third stage larvae (P>0.001). Maximum rate of reduction in number of infective larvae of H. contortus were observed with addition of 100000 conidia of isolate No. 1 (96.65%), isolate No. 19 (94.64%) and isolate No. 49 (94.96%).

Conclusion: In recent years, isolation of types of nematophagous fungi especially A. oligospora were reported in different regions of world. They could cause significant reduction in number of infective larvae of nematodes and also free-living nematodes by addition of different amount of fungal conidia. Our results were showed in regions with moist weather and adequate moisture in Iran, A. oligospora may be present and also these nematophagous fungi can be used for controlling larvae of H. contortus and other gastrointestinal nematodes (Trichostrogylids) in vitro condition.

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403 (615)
FIELD EFFICACY OF A VACCINE AGAINST NEOSPORA CANINUM IN MEXICO
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Neospora caninum affects several species producing great economical loss. To evaluate the field efficacy of a vaccine of Neospora caninum (Bovilis NeoGuard), a study was performed to evaluate the serological response and the rate of reduction of abortions in dairy cattle in Mexico.

A dairy herd having a history of abortions was selected. Evaluation of seroprevalence using indirect ELISA was used for Neospora caninum, Bovine virus diarrhea, Bovine Herpes virus 1, Parainfluenza 3 virus, Leptospiira hardjo and Brucella abortus. The criteria for the study were the presence of dogs, abortions and positive seroprevalence to Neospora caninum. 200 cows were selected at random on the 3rd month of pregnancy and separated into two groups of 100 animals each (vaccinated and placebo). First bleeding was done of cows on each group previous to first vaccination, followed by a second bleeding four weeks later, previous to second vaccination and a third bleeding four weeks later. Analysis of variance and Chi-square were used to identify significant differences. Five out of 9 fetuses with histopathological lesions of microgliosis and multifocal necrosis, lymphohytic periporal hepatitis, necrotic encephalitis, necrotic lymphocytic miosis and interstitial nephritis, suggested the diagnosis of Neospora caninum confirmed by immunohistochemistry. There was no difference in seroprevalence between vaccinated and placebo groups in the first bleeding. Statistical difference was found (p<0.05) between first and second bleedings and second and third bleedings in vaccinated animals. No difference was found in the placebo group. A difference was observed between second bleedings and third bleedings between both groups. A total of 41 abortions were observed out of 200 cows. There were 12% and 29% abortions in cows from the vaccinated and placebo groups respectively, with an average of 89 days of abortion for both groups. No statistical difference was found between vaccinated and placebo groups for the other 5 infectious agents. There was a savings of 13,000 USD, due to vaccination. The vaccine was effective in reducing abortion caused by Neospora caninum in cattle.

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404 (1957)
ACTIVITY OF A LONG ACTING FORMULATION (IVERMECTIN + ABAMECTIN) AGAINST BOOPHILUS MICROPLUS ON NATURALLY INFESTED BOVINE HOSTS
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The macrocyclic lactones molecules have become, due to their high efficacy and ease of application, often the preferred treatment for the control of the cattle tick, Boophilus microplus. The continue expose of these ticks to the macrocyclic lactones has led to resistant populations. Hence a constant search for new alternatives to control B. microplus is required. As such a novel innovative long action formulation containing 2.25% ivermectin + 1.25% abamectin*, was developed and its efficacy in tick control was investigated. The performance of this new formulation was compared with a 3.15% ivermectin** formulation and a control group. After subcutaneous treatment of the animals (1mL/50kg), female B. microplus counts were performed on days 1, 3, 7 and weekly afterwards, until the 91st day post-treatment (DPT). The macrocyclic lactones association significantly reduced (P<0.05) the number of female ticks, when compared to the untreated control group, from the 7th to the 84th DPT. On the 14th DPT, the ivermectin + abamectin association* showed a 92.98% efficacy in tick control, attaining 99.63% of efficacy on the 21st DPT, and maintaining rates over 98% until the 42nd DPT. On the 63rd DPT the new formulation showed a 91.61% efficacy, which decreased to 70.37% on the 77th DPT. The B. microplus control of the avermectin association*, compared to that of the 3.15% ivermectin**, was not statistically different (P>0.05) during the whole experimental period. However, the tick control of the 3.15% ivermectin** differed from the control group only until the 70th DPT.

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405 (2053)

EFFECT OF A 3.5% IVERMECTIN + ABAMECTIN ASSOCIATION ON WEIGHT GAIN OF NELORE CALVES KEPT ON PASTURE

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Parasite control is an important aspect in today’s cattle production, and has significant impact on the revenues
of the farm. Among all known parasiticides, the avermectins are the most used today, primarily because of
their long acting effects and the ease-of-application. This study evaluated the weight gain of recently weaned
Nelore calves, kept on Brachiaria decumbens pasture, treated with the following formulations: GI: 2.25%
ivermectin + 1.25% abamectin*, GII: 3.15% ivermectin** and GII: untreated controls. Prior admission all
animals were confirmed positive for nematode infections by Haemonchus spp, Cooperia spp and
Oesophagostomum spp. All products were administered subcutaneously, at a 1 mL/50 kg dosage, on two
experimental dates (days zero and 60). To assess weight gain, animals were weighted individually on days -
30, zero, 30, 60, 90 and 120. Twelve hours prior to weighing animals were restricted of water and food. Fecal
samples were collected on all observational dates for measurement of parasite burden by counting of eggs
per gram of feces (EPG). At the end of the study (120 days) the calves treated with the 3.5% ivermectin +
abamectin combination* (GI) showed lower EPGs and gained significantly more weight (9.16 kg) compared to
those treated with 3.15% ivermectin** (GII) and an additional 29.17 kg compared to untreated animals (GIII).
Thus, treatment with ivermectin + abamectin association* promoted a better weight gain in calves when
compared to the 3.15% ivermectin** treatment.

* Solution 3.5% LA - Akzo Nobel Ltda - Intervet Division.
** Commercial product purchased on the market.

406 (5062)

BOVINE BESNOITIOSIS, ONE APPROACH FOR A BETTER UNDERSTANDING OF ITS IMPORTANCE IN PORTUGAL

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Bovine besnoitiosis has a wide geographical distribution and, in Africa, where severe clinical forms are
frequent, it leads to important economical losses. The disease was identified in Portugal in the late XIX
century, as referred by Borges in 1912. However, only in the late eighties of XX century, the disease gained
relevant scientific attention and, at present, besnoitiosis is known to occur in a larger geographic scale.
In this work we assessed the prevalence of infection on a herd basis. Animals were followed for the
manifestation of clinical signs; serum samples were tested by indirect immunofluorescence for the presence
of specific antibodies and skin biopsies were tested by PCR and histopathology for the presence of Besnoitia
besnoiti. In a herd of 326 animals, where during the last year 12 animals showed clinical manifestations of
Besnoitiosis, all the animals were tested by the three methods. Although detection of the agent was achieved
only in a few animals, IFI revealed specific antibodies in 36% of the population.
Overall our results show expressive prevalence rates, which may have a high economical importance,
particularly in herds where veterinarians and farm workers are not alert for the dramatic clinical signs of the
disease. In addition, they strongly suggest that this disease has important economical consequences due to
high losses in body condition and to the fact that sick males are invariably infertile, leading to a higher level of
losses through a small output of the most important product in a beef cattle industry: the calf.

Funding: CIISA, ICAM

407 (1436)

RECENT OBSERVATIONS ON BOVINE DICROCOELIOSIS IN FRANCE: PREVALENCE, DIAGNOSIS,
PATHOGENICITY AND TREATMENT

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Dicrocoelium dendriticum (small liver fluke) is a common parasite of ruminants, which has been well studied in
sheep but is not well known in cattle. It has been reported to have a low prevalence and a low pathogenicity in
cattle. Parasitic infestation with Dicrocoelium dendriticum has been observed for many years in beef cattle
herds in the Charolais region of France and many recent observations (necropsy findings, slaughterhouse
liver condemnations) suggest that this parasitic disease in cattle is underestimated by veterinarians and
PREVALENCE OF GIARDIA DUODENALIS IN FRENCH BEEF FARMS AND CALF REARING UNITS

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The awareness of Giardia duodenalis infections as a threat to public health has increased significantly over the years. The aim of this study is to evaluate the prevalence of G. duodenalis infections in range beef calves in France and to establish its excretion pattern. In addition the presence of G. duodenalis cysts in drinking water was investigated. Fecal samples were collected of 75 Angus and Angus cross cattle were identified as being positive for antibodies to Anaplasma marginale utilizing ELISA during a herd screening. Approximately 5 months following initial ELISA testing, all 75 cattle were tested again with ELISA and PCR. Sixty-five cattle remained ELISA and PCR positive, confirming them as Anaplasma marginale carriers. Twenty-five cattle were randomly selected and treated with 60 ml (4.5 ml per 45 kg) of long acting oxytetracycline subcutaneously on day 0. Five cattle were randomly selected and used as untreated controls. On days 3 and 6, the treated cattle were again injected with 60 ml of long acting oxytetracycline subcutaneously. On day 14, all cattle were tested utilizing PCR. All 5 untreated control cattle were positive. Of the treated cattle, 25 of 28 were PCR positive. Two treated cattle were not tested. On day 74, all cattle were tested utilizing ELISA and PCR. All 5 untreated controls remained PCR positive while 2 of 5 remained ELISA positive. Of the treated cattle, 24 of 29 remained PCR positive and 20 of 29 remained ELISA positive. One treated cow was not tested. Three injections of long acting oxytetracycline subcutaneously at the labeled dose, was not successful in eliminating Anaplasma marginale in these carrier cattle when evaluated by ELISA or PCR testing methods.

Funding: Pfizer, France
The prevalence in range beef calves was 78.8% (CI at 95% [73.3; 82.7]) the prevalence for their dams was 32.6% (confidence interval at 95% [27.7; 38.3]). The highest excretion patterns were observed in calves 3 and 4 months of age, the time of natural weaning. 44% of drinking water samples were positive for Giardia cysts with an average of 101 cysts per 100 liters. The results clearly demonstrate that the majority of calves between 1 and 4 months secrete Giardia cysts in the environment, while also the dams of these calves pose a threat to non-infected animals. The identification of genotype assemblage A, infecting humans and cattle, suggest a real zoonotic risk.

411 (2560)
CONTINUOUS RELEASE BOLUS VERSUS POUR ON APPLICATION OF ANTHELMINTIC DURING FIRST GRAZING SEASON: COMPARISON OF GROWTH PERFORMANCE AND EGG SHEDDING

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This multicentric randomized controlled trial aims at comparing the shedding of nematode eggs in faeces and the average daily weight gain of cattle during the first grazing season with two nematode control strategies: topical treatment at turnout and eight weeks later, or application of a continuous slow release intra ruminal device at turnout. For this study 147 heifers and 24 steers were selected from 9 farms in France. Animals were randomly allocated to one of three treatment groups. Group 1 was given a fenbendazole slow release bolus (Panacur® SR bolus). Group 2 was given doramectin topically at 500 μg/kg while Group 3 was left untreated. All cattle of a farm were weighed and then turned out on the same pasture on day 0. No particular precaution was taken to prevent grooming between pour on treated animals or within groups. On Day 56, all animals were weighed and faecal samples collected for faecal egg counts (FEC) and group 2 animals were treated again. At winter housing, all animals were weighed again and faecal samples collected.

The three groups were not statistically different in terms of bodyweights on day 0 (mean = 253 kg). Animals were grazed from May to October/November 2002 (average of 177 days on pasture). Two animals from the control group were given an emergency anthelmintic treatment in September (fenbendazole oral suspension). Considering the entire grazing season, there was a trend that growth performance in Group 1 was better than Group 2. However, this difference was not statistically significant (p=0.18, one-way Anova followed by Bonferroni), except for one farm in Normandy (p = 0.003). Larval culture and differentiation showed a parasitic challenge with primarily Ostertagia ostertagi and Cooperia spp (predominant in four sites). The difference of FEC between group 1 and the other two groups was statistically significant on day 56 (p<0.001). The continuous release bolus group showed the lowest FEC at day 56 (hence a very moderate nematode infection). On the other hand, the 0-8 weeks endectocide program, as assessed by FEC and average daily weight gain, appeared to be less effective under the local conditions and with a risk of non compliance by farmers, since animals need to be collected and re-treated in the middle of the long grazing season.

To apply a fenbendazole intraruminal bolus is an easy and single shot operation that allows the build-up of immunity while enhancing production.

412 (2551)
PREVALENCE OF GIARDIA DUODENALIS IN FRENCH DAIRY FARMS AND CALF REARING UNITS

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In various countries, Giardia duodenalis is a well know protozoan parasite responsible of intestinal infection in humans and animals. The transmission of the parasite from animal to human, mainly by drinking water, is a major public health problem. The aim of this study was to evaluate Giardia duodenalis prevalence in calves and to investigate the excretion pattern of cysts.

Faecal samples were collected from 43 dairy farms (n=299) and 40 veal farms (n=280) in the “Pays de la Loire” area. Of each farm approximately 7 samples were collected from 1 to 6 months old calves. Samples were analysed by the direct immunofluorescent method. On each farm animals excreting Giardia duodenalis were found. 206 out of 299 calves in dairy herds excreted Giardia's cysts (68.9%, CI at 95% [63.6; 74.1]). On veal farms, 257 out of 280 calves were found positive (91.8%, confidence incidence at 95% [88.6; 95]).

Calves were allocated to four excretion groups: 6.3% of dairy calves and 14.3% of the veal calves excreted over 10000 cysts per gram of faeces. The maximum number of excreted cysts per gram was around 10000 cysts in dairy farms and 400000 in veal calves units. The excretion peak was seen at 1 month in dairy calves and between 1 and 2 month in veal calves. The observed prevalence of excretion is comparable with results obtained by Olson et al. (1997) in British Colombia. In this survey the detection method and the age of the calves sampled was similar.

Cyst excretion waned in older calves probably because of immunity development. The higher excretion patterns observed in veal calves compared to dairy calves may be linked to a higher population density, a more stressful environment and a deficient immunity (McDonough and al., 1994).
413 (2066)
EFFICACY OF A 2.25% IVERMECTIN + 1.25% ABAMECTIN ASSOCIATION AND ITS RESIDUAL ACTIVITY AGAINST THE CATTLE TICK BOOPHILUS MICROPLUS (ACARI:IXODIDAE) IN A STALL TEST
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The efficacy of a new long acting formulation containing 2.25% ivermectin + 1.25% abamectin* was evaluated and compared with that of a 3.15% ivermectin, in two stall tests using cattle experimentally infested by B. microplus (São Gabriel Strain). Both treatments were administered subcutaneously at a 1ml/50kg dosage. In the first study the associated avermectin formulation showed 87.3% efficacy on the 4th day post-treatment (DPT), and attained its highest efficacy on the 9th DPT (99.5%). These results were maintained up to the 26th DPT. Comparing the two treatments, there was no statistical difference (P>0.05) in efficacy. In the second study, the efficacy of the avermectin combination* was higher on the 4th DPT when compared to the 3.15% ivermectin** formulation (94.5% and 78.9%, respectively). In the ivermectin + abamectin association efficacy was 94% on the 5th DPT and above 97% from the 8th DPT to the final observation date. Efficacy in the group treated with 3.15% ivermectin** was 91.5% on the 5th DPT, and above 97.7% from the 9th DPT onwards to the end of the study. Statistical comparison showed no differences between the two formulations under investigation. After weekly artificial tick infestations, the presence of engorged female ticks was seen only after the 9th week in both groups. At the 70th DPT a mean number of 6 female ticks was counted for the group treated with the ivermectin + abamectin* combination, while on the group treated with the 3.15% ivermectin formulation, the average number was 16.

* Solution 3.5% LA - Akzo Nobel Ltda - Intervet Division.
** Commercial product purchased on the market.

414 (3036)
KILLED WHOLE NEOSPORA CANINUM VACCINE INDUCES AN IMMUNE RESPONSE SIMILAR TO THAT FROM NATURALLY INFECTED PREGNANT HEIFERS
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Chronically infected cattle have protective immunity against a second N. caninum exposure. The objective of this study was to compare the immune response to N. caninum in naturally, chronically infected heifers and vaccinated heifers. Three N. caninum seropositive heifers, which were born from seropositive dams, and 6 seronegative heifers born from seronegative dams were used in the study. All Holstein heifers aged 24-36 months were pregnant. Humoral and cell mediated immune response were evaluated during the second third of the gestation (week 13, 15, 17, 19, 21, 23, 25 of pregnancy). Humoral immune response was investigated by indirect fluorescent antibody test (IFAT) and indirect ELISA for detecting isotype specific antibodies, and cellular immune response was determined by specific lymphocyte proliferation and interferon-γ assays. A killed whole N. caninum tachyzoite preparation containing 45 mg of protein/5 ml of dose was formulated with 70% of mineral oil adjuvant (13% of Arlacel C, 85% of Marcol 52 and 2% of Tween 80). Additionally, an immunomodulator (RN-205) containing bacterial lipopolysaccarides was inoculated. Four seronegative heifers were immunized with N. caninum tachyzoite preparation and RN-205 during week 13, 15 and 17 of gestation. Two seronegative heifers inoculated with adjuvant and RN-205 were used as negative controls. N. caninum-specific antibody responses increased in immunized cattle by week 15 post-vaccination (p.v.) (mean reciprocal antibody titres 450 ± 252), peaked at week 23° p.v (mean 16000 ± 6400). Maximum antibody response in naturally infected heifers was observed at 19° week of gestation (mean: 3467 ± 2810). The overall mean serum IFAT titres were significantly higher (P < 0.05) in immunized heifers compared with naturally infected heifers from week 17 to week 25. Analysis of isotype specific antibodies revealed a predominant IgG2 response in 2 infected heifers and a predominant IgG1 response in the other one. Similar amounts of IgG1 and IgG2 were found in 3 immunized heifers; however a predominant IgG2 response was developed in one immunized heifer from week 19 to week 25. Control heifers remained seronegative through the study by IFAT and ELISA. N. caninum-specific cell-mediated immune response was only increased in naturally infected cattle by week 19 of gestation (P < 0.05). Peripheral blood mononuclear cells from immunized animals produced similar interferon-γ concentrations of (P >0.78) infected animals.

415 (2408)
STUDY ON THE EFFICACY OF TOLTRAZURIL (BAYCOX 5%, ORAL SUSPENSION) IN AN INFECTION MODEL WITH EIMERIA ZUERNII - DOSE CONFIRMATION
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The objective of this study was to confirm the full efficacy of toltrazuril at a dose of 15 mg/kg administered orally in an experimental Eimeria zuernii infection. 24 bull calves aged from ten days to four weeks were each infected with 1.5 x 10^5 sporulated Eimeria zuernii oocysts. On day 14 p.i. (post infection) the calves passing...
the entry criteria were randomly assigned to two groups and treated orally as follows: group A (n=11): 20 ml water (untreated control), group B (n=12): 15 mg/kg toltrazuril. The calves were clinically monitored on a daily basis; faecal samples were taken three times weekly during the first two weeks post infection and daily thereafter in order to determine faecal consistency and oocyst excretion. The calves in the untreated group developed clinical coccidiosis as a result of the infection. The groups differed considerably both with regard to the duration and the severity of diarrhoea. Faecal consistency showed statistically significant differences between the groups: in group A, 40% of faecal samples tested between day 14 and day 32 p.i. were altered (diarrhoea), in group B the proportion was 3%. Watery or bloody diarrhoea, i.e. severe lesions, were only observed in group A. Patency in group A started on day 17 and day 24 p.i.; each calf in this group shed oocysts for several days (up to 123,000 oocysts per gram faeces). In group B only one animal passed 50 opg on day 26 p.i., while the remaining calves in this group did not shed Eimeria zuernii oocysts at any time during the study.

416 (2433)

STUDY ON THE CONTROL OF E. ZUERNII COCCIDIOSIS IN CALVES WITH A 5% SUSPENSION OF TOLTRAZURIL UNDER FIELD CONDITIONS
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A blind, randomized field study was conducted in a herd with naturally occurring calf coccidiosis caused by Eimeria zuernii to check the efficacy of a single dose of toltrazuril (Baycox 5% Oral Suspension) of 15 mg/kg liveweight. Three groups of 24 calves each were formed (in two successive sequences with 12 calves per group) and loose-housed in groups. Group A served as the placebo-treated control. The efficiency of a metaphylactic treatment (one week before the anticipated onset of scouring, group B) and a therapeutic toltrazuril treatment (after onset of clinical symptoms of coccidiosis, group C) was tested. Coccidiosis-induced scouring occurred from day 21 after initial exposure (calves moved to a contaminated shed). 58% of the calves in group A developed diarrhoea. Diarrhoea was diagnosed in 8.6% of cases, based on all 840 samples taken during the study period. 96% of the calves in group A excreted Eimeria zuernii oocysts on at least one day in the course of the study. In the therapy group (group C) the proportion of calves with coccidiosis-induced diarrhoea was reduced to 29%; in this group 5.5% of all samples during the study period were diarrhoeic. The oocyst excretion rate at 87.5% was almost as high as in group A. The metaphylactic treatment on the other hand controlled clinical coccidiosis completely. There was not one case of Eimeria zuernii-associated diarrhoea in group B, although oocysts were detected in the faeces of 15 out of 24 calves (62.5%) at some time during the study. The metaphylaxis group, unlike the therapy group, differed significantly (p<0.01) from the control group with regard to scouring and oocyst excretion. The selected dose of 15 mg toltrazuril/kg liveweight controls calf coccidiosis in the field efficiently. Although the therapeutic treatment showed some benefit, the preventative approach is definitely preferable in herds where the epidemiological situation is clear.

417 (2436)

STUDIES ON THE EFFICACY OF TOLTRAZURIL (BAYCOX 5%, ORAL SUSPENSION) IN AN INFECTION MODEL WITH EIMERIA BOVIS - DOSE DETERMINATION AND DOSE CONFIRMATION
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The objective of the studies was to determine a fully effective therapeutic dose of toltrazuril following experimental Eimeria bovis infection in calves. First, a dose determination study (study 1) was conducted with four treatment groups (A: untreated, B: 5 mg/kg toltrazuril, C: 15 mg/kg toltrazuril, D: 25 mg/kg toltrazuril) following infection with 5 x 10^4 sporulated Eimeria bovis oocysts per animal (n = 8-9 calves/group). In the subsequent dose confirmation study (study 2), 18 bull calves aged from two to four weeks were each infected with 1 x 10^5 sporulated Eimeria bovis oocysts. On day 14 p.i. (post infection) the calves were randomly assigned to two groups and treated as follows (n = 9 calves/group): group E: untreated, group F: 15 mg/kg toltrazuril orally. In both studies the calves were clinically monitored on a daily basis; faecal samples were taken three times weekly during the first two weeks post infection and daily thereafter in order to determine faecal consistency and oocyst excretion. The calves were weighed once a week. The animals in the untreated group developed clinical coccidiosis as a result of the infection. The groups differed with regard to both the duration and the severity of diarrhoea. Faecal consistency showed statistically significant differences between the groups: in group E, 87% of faecal samples tested were negative, in group F the proportion was 26%. Patency in group E started on day 18 or 19 p.i. and reached the highest mean of 58256 opg (oocysts per gram faeces) on day 21 p.i.; this group was shedding oocysts continuously throughout the study. In group F, low oocyst shedding was observed in one calf from day 20 p.i. to day 23 p.i. and in another calf on day 20 p.i., while the remaining calves in this group did not shed Eimeria bovis oocysts at any time. The weight development of the calves also differed significantly, with the treated animals showing distinctly better weight gains.

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HISTOPATHOLOGICAL STUDY OF THE INTESTINAL MUCOSA OF CALVES AFTER EXPERIMENTAL INFECTION WITH EIMERIA BOVIS - COMPARISON BETWEEN UNTREATED CALVES AND CALVES TREATED WITH TOLTRAZURIL (BAYCOX 5%, ORAL SUSPENSION) AT DIFFERENT TIMES

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The objective of the study was to demonstrate histopatho-logical lesions in the intestines of calves after an experimental infection with Eimeria bovis oocysts. To this end, untreated calves and calves treated with toltrazuril at different times post infection (p.i.) were tested. A total of 24 bull calves aged from two to four weeks were each infected with 1 x 10⁵ sporulated oocysts of Eimeria bovis and treated orally as follows (n = 8/group): Group A received 15 ml water on day 12 p.i. (untreated control), group B received 15 mg/kg toltrazuril on day 12 p.i. and group C was treated with 15 mg/kg toltrazuril after the onset of clinical symptoms (day 18 p.i.). Faecal samples were tested (for faecal consistency and oocyst excretion) three times weekly from the day of infection to day 12 p.i. and then daily until completion of the study (day 35 p.i.). Pathological examinations were performed at three different times: Groups A and B: days 16 (prepatency), 20 (patency) and 28 (late patency/postpatency) p.i., group C on days 20, 28 and 35 p.i.

The untreated control calves (group A) developed clinical coccidiosis at the end of the prepatent period, manifested by diarrhoea and oocyst excretion. Necropsy revealed typical signs of severe intestinal inflammation like reddening and fibrin-like covering of the mucosa. Histologically, all animals showed the presence of oocysts in the intestinal epithelia. They occurred in the caudal jejunum, rarely in the ileum, and were most pronounced in the cecum and colon. Severe signs of inflammation in terms of acute typhlitis and colitis as well as pseudo-membrane formation and inflammatory demarcation in the submucosa were seen in nearly all animals. The clinical symptoms were distinctly reduced in the calves in group B. Nearly no pathological findings were observed at necropsy. Histologically, with one single exception, all intestinal localizations were free of any parasitical structure. Only some granulocytic or mixed cellular inflammatory infiltrates were seen in the mucosa. Cellular debris occasionally was found in the crypts and glands.

Treatment at the end of the prepatent period (group C) produced no clinical response, as was to be expected.

Results from necropsy and histopathology were comparable as described for group A, too.

ANTHELMINTIC EFFICACY OF A NEW LONG ACTING FORMULATION CONTAINING 2.25% IVERMECTIN + 1.25% ABAMECTIN, ON THE TREATMENT OF CATTLE NATURALLY INFECTED BY NEMATODES

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Like for most other anthelmintics, parasite drug resistance has also developed against acrocyclic lactones, over the years. This resistance, which is most evident with ivermectin, is said to be induced by sub-optimal dosing and its frequent application. Recent studies in Brazil and other countries have reported the existence of Cooperia spp. and Haemonchus spp. populations resistant to ivermectin and doramectin. In this study the anthelmintic efficacy of two long acting formulations: 2.25% ivermectin + 1.25% abamectin* and 3.15% ivermectin** was compared in naturally infected bovine hosts. For both formulations, on the 13th day post-treatment (DPT) EPG counts were reduced in 100.0% and 69.33%, respectively. On the 14th DPT, 18 cattle (allocated in three groups of six animals each) were necropsied for assessment of nematode burden. Eight nematode species were identified. Both formulations showed a 100% efficacy against Cooperia pectinata, Trichostrongylus axei, Oesophagostomum radiatum and Dictyocaulus viviparus. The ivermectin + abamectin association* showed a higher anthelmintic efficacy than 3.15% ivermectin** against Haemonchus placei, C. spatulata, C. punctata and Trichuris discolor.

* Solution 3.5% LA - Akzo Nobel Ltda - Intervet Division.
**Commercial product purchased on the market.

PRACTICAL USES OF DECOQUINATE IN PREGNANT FEMALES TO CONTROL CRYPTOSPORIDIOSIS INFESTATIONS IN NEWBORN CALVES AND LAMBS

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Cryptosporidiosis causes severe diarrhea and frequently death in newborn calves and lambs. Treatment at the end of the prepatent period (group C) produced no clinical response, as was to be expected.

Results from necropsy and histopathology were comparable as described for group A, too.

420 (1204)
DECOCQUINATE IS A NON ANTIBiotic SYNTHETIC MOLECULE, ACTIVE ON CERTAIN PROTOZOAA - COCCIDIA, TOXOPLASMA, CRYPTOSPORIDIA, NEOSPORA - WHICH WAS GRANTED A ZERO-WITHDRAWAL PERIOD IN FRANCE FOLLOWING ITS CLASSIFICATION IN PRACTICAL USES OF DECOQUINATE IN PREGNANT FEMALES TO CONTROL CRYPTOSPORIDIOSIS INFESTATIONS IN NEWBORN CALVES AND LAMBS

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Decoquinate is a non antibiotic synthetic molecule, active on certain protozoa - coccidia, toxoplasma, cryptosporidia, Neospora - which was granted a zero-withdrawal period in France following its classification in

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Annex II for MRL. Since 1994, on more than 12,000 cows, a prevention treatment was successfully applied on Charolais beef cows in order to reduce oocyst shedding at parturition. Cows received 1.25 mg decoquinate / kg BW / day for 30 days prior to calving and for 8 days following it in the form of a supplemented feed. This treatment was regularly associated with the disappearance of clinical signs on calves.

In order to specify and confirm this protocol, the same prevention was applied in ewes but with a higher dosage (as for lamb coccidiosis 1mg decoquinate / kg BW is indeed recommended as compared to 0.5 in calves). Using this supplemented feed on thousand of ewes over several years led to the identification of 2 key points for success:

- The dosage must be strictly respected: underestimation of the weight of pregnant ewes and/or overestimation of feed consumption was responsible for failures.
- The treatment must be done before and after parturition: first parturition ewes who lamb 3 weeks after multiparous ewes (in the Roquefort area) were treated at the same time for practical reasons, i.e. at the end of their pregnancy but not exactly at lambing. The lambs from these 1st parturition ewes were noted several times as clinically affected.

In conclusion, large scale ewe trial results have confirmed those results obtained in beef cows and underscore the effectiveness of the treatment of pregnant mothers as a mean to control the spread of cryptosporidiosis to newborn ruminants.

421 (2077)
THERAPEUTIC EVALUATION OF A 2.25% IVERMECTIN + 1.25% ABAMECTIN ASSOCIATION ON THE TREATMENT OF BOVINE HOSTS NATURALLY INFESTED BY DERMATOBIA HOMINIS (LINNAEUS JR., 1781) (DIPTERA:CUITEREBRIDAE) LARVAE
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Myiases by Dermatobia hominis larvae are of great economical importance in Latin America's cattle production and have been the subject of much research to attain new formulations for better control of this problem. In this view, the efficacy of a new long acting formulation, based on avermectins (ivermectin + abamectin) against Dermatobia hominis larvae, was evaluated using naturally infested bovine hosts. A comparative study was executed using four groups of 10 bovine hosts each. Group I (GI) was treated with the formulation containing 2.25% ivermectin + 1.25% abamectin*, GIi with 3.15% ivermectin**, GIi with 1% doramectin*** and the control group, GIV, with saline solution. Medication was administered subcutaneously, at a 1 mL/50 kg dosage. Results showed a 100% efficacy from the 14th to the 91st day post-treatment (DPT) for GI and GIi, and from the 35th to the 91st DPT for the 1% doramectin** formulation. Although there was no statistical difference (P>0.01) when comparing results from different treatments, the mean larvae number for GI, treated with the macrocyclic lactone association, was the lowest on the last day of observation.

* Solution 3.5% LA - Akzo Nobel Ltda - Intervet Division.
** Commercial product purchased on the market

422 (2880)
CROSS-SECTIONAL STUDY OF NEOSPORA CANINUM IN URUGUAYAN DAIRY CATTLE
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Neosporosis or Neospora caninum is a recently discovered disease but widely spread around the world, that it is considered an important cause of infectious abortion in dairy cattle. A serologic study was conducted to determine the seroprevalence of Neospora in lactating dairy cows, in the most important dairy region of Uruguay.

The first diagnosis of Neospora in cattle in the country was published at July 1999. Until now, seroprevalence data in Neosporosis in dairy herds was unknown. The objective of this study was to estimate the serologic prevalence and the spread of the disease among herds. The dairy population in the study region is 2,000 herds with 180,000 milking cows. In 2002, two step random samples were drawn from the population. In the first step 42 dairy herds were selected and in the second step 20 milking cows were selected in each farm by systematic sampling. The total number of studied cows was 844. Serum-prevalence in cows was estimate weighting by farm population (number of cows) using the routine of Intercooled Stata 7.0. Sera were tested with an ELISA kit from Bomeml Chekits. The seroprevalence estimated for the population was 16.0% ± 2.9%. A positive herd was defined as any herd with one or more ELISA positive cows. The proportion of positive herds was 97.6% (41/42). Most of the dairy farms (90.5%) have a low prevalence with less than 30% positive cows. In conclusion, the disease is present in Uruguay, and it is high spread among farms. Therefore, it is recommended starting developing control programs in the country.

423 (2039)
THERAPEUTIC AND RESIDUAL EFFICACY OF AN IVERMECTIN + ABAMECTIN ASSOCIATION AGAINST BOOPHILUS MICROPLUS IN EXPERIMENTALLY INFESTED CATTLE
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The cattle tick Boophilus microplus is the most economically important external parasite in cattle production today. The tick is widely spread throughout Brazil and causes losses up to two billion dollars each year. In this study, the therapeutic and residual efficacy of a long acting formulation containing 2.25% ivermectin + 1.25% abamectin* in the treatment of cattle experimentally infested by B. microplus (Mozzo strain) was evaluated. This new formulation was compared with an existing 3.15% ivermectin** formulation. Infestations were performed in 15 male bovine hosts, using 5000 Boophilus microplus larvae (Mozzo strain), three times a week. On day 0 of the study, the animals were distributed in three groups of five animals each. The groups were allocated to one of the following treatments: GI: 2.25% ivermectin + 1.25% abamectin*, GII: 3.15% ivermectin** and GIII: control animals treated with saline solution. All medications were administered subcutaneously at a 1 mL/50 kg dosage. Detached female ticks were collected and counted from each host up to the 80th day post-treatment (DPT). Both formulations showed maximum efficacy (100.0%) for 26 and 27 experimental dates respectively, and both demonstrated good efficacy (>80%) against B. microplus until the 70th DPT. Statistical analysis showed no difference between treatments (P>0.05) for the duration of the experimental period. Therefore, the long residual activity of both formulations against B. microplus was confirmed.

* Solution 3.5% LA - Akzo Nobel Ltda - Intervet Division.
** Commercial product purchased on the market

424 (3276)
GRAZING MANAGEMENT AND PARASITIC DISEASES IN ORGANIC DAIRY HERDS
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A substantial proportion of Danish dairy herds are certified organic. The organic production system aims at having all cattle on pasture during summertime and restricts use of external inputs, e.g. the prophylactic use of anthelmintics. The aim of this study was to collect data on grazing management procedures in organic dairy herds, to assess the level of parasitic diseases and to identify risk factors. A questionnaire was sent in fall 2002 to all Danish certified organic dairy farmers (n=717) asking about farm size, grazing management in 2002 and diagnosed/treated cases of parasitic disease in all age groups. 293 (41%) questionnaires were returned and used. The average farm size was 120 ± 60 ha of approx. 47% clover-grass within the rotation and 9% permanent pasture. Average number of cows was 90 ± 40 with a mean yield of 7505 ± 950 kg ECM. Diarrhoea was observed in first-grazing-season heifers (FGSH), second-grazing-season heifers (SGSH) and cows on 35%, 3% and 5% of farms in 2002, which reflected the number of farms diagnosing gastrointestinal nematodes (GIN): 26% in FGSH and 9% in SGSH. Coughing was observed on 47%, 36% and 9% of the farms in FGSH, SGSH and cows. Coccidia infections were diagnosed in FGSH on 13% of the farms. Among FGSH lungworm disease was diagnosed and treated with anthelmintics on 16-27% of the farms in each season, 1998-2002. Similar figures for SGSH and cows were 6-14% and 2-3%. 10% of the farms diagnosed lungworm repeatedly in all 5 years in the dairy herd whereas 30% of farms did not diagnose lungworm at all. Lungworm infections were diagnosed more often in both age groups on a farm than expected from the incidence of each of the age groups (p<0.0001). FGSH turned out on pasture grazed previously by cattle and not moved during the grazing had a higher risk of diagnosis of GIN (p<0.05). Unexpectedly, move/moves to clean pasture during the season increased the risk of GIN in FGSH (p<0.0001), which could reflect a disease treatment instead of preventive use. The majority of diagnosed cases of GIN, coccidia and lungworm were treated with antiparasiticides whereas few treatments were prophylactic. There is no connection between the farmer's attitude to organic/conventional agriculture and their use of parasite control. The study thus confirmed that GIN are mainly a problem of FGSH whereas lungworm disease is found in all age groups. Relatively many farms had lungworm infections repeatedly. Associations between grazing management and risk of parasitic diseases were difficult to find.

425 (3483)
A STUDY TO INVESTIGATE THE INFLUENCE OF BOVINE VIRUS DIARRHOEA ON PREGNANT HEIFERS INFECTED WITH NEOSPORA CANINUM AND THE FIRST IDENTIFICATION OF CIRCULATING N. CANINUM DNA IN CATTLE
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A study was designed to challenge with BVD virus, 6 seronegative and 6 previously N. caninum seropositive pregnant heifers which were seronegative to BVD. A similar 12 control heifers (6 seropositive and 6 seronegative) were infected with BVD virus, 6 seronegative and 6 previously N. caninum seropositive.
seronegative to N. caninum) were to be kept at a different site unchallenged by BVD. When the heifers arrived at the experiment site, 6 of the seropositive heifers had aborted. An experienced veterinarian had confirmed pregnancy 6 weeks previously. Heifers underwent blood sampling on arrival at the experimental sites and weekly thereafter. Sera were examined for antibody to N. caninum using immunofluorescent antibody testing (IFAT). A PCR conducted on the whole blood of the heifers demonstrated the presence of N. caninum nucleic acid in the blood.

Some pregnant heifers were re-allocated to treatment groups because 6 of the Neospora seropositive heifers had aborted. Four of the 6 pregnant Neospora seropositive heifers remaining were challenged with BVD virus while 2 were not challenged. Six of the Neospora negative heifers were challenged with BVD while 6 remained as controls.

Serology showed that all 6 heifers that aborted before arrival were seropositive to N. caninum as were 2 other previously seropositive animals and a previously seronegative animal. A PCR conducted at arrival showed circulating DNA in all previously seropositive animals plus two previously seronegative animals (one of which was now seropositive). Repeated serological testing on sera and PCR testing on whole blood revealed that all animals that had aborted remained seropositive and had circulating N. caninum DNA until approximately 7 to 11 weeks after sampling began at 1 to 6 weeks after abortion. Surprisingly, serial PCR on the blood of 12 previously seronegative heifers revealed N. caninum DNA associated with low or intermittently high antibody titres to N. caninum in 11 of them when this would not have been expected based on previous serology. No heifers challenged with BVD virus in the experimental period aborted. Circulating N. caninum DNA was identified in the blood of all aborting and previously seropositive heifers, plus two previously seronegative ones. Heifers that were previously seronegative later had circulating DNA in association with low or intermittently high serotoxicities. The relationship of infection status and serology need further investigation since prior negative serological results did not predict infection status reflected by circulating DNA.

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426 (831)
EFFECT OF GROWTH HORMONE (BST) ON SEMEN PRODUCTION IN BULLS
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The global objective of the present work was to verify the effect of prolonged treatment of bulls with growth hormone Bovine Somatotropin (Posilac) on semen production. Twelve (12) mature bulls (between 5 and 10 years old) of Holstein breed, placed under same environment condition were injected twice monthly with 100mg of Posilac s-c for 26 weeks. Thirteen weeks after the first injection, 100µg of GnRH (Factrel) were injected s-c to each bull. Semen and collection were performed starting four weeks before beginning of the treatment and up to 42 weeks after. Parameters that were looked for included volume of each ejaculate, total sperm output per collection, IGF-1 serum concentration. Data of semen production were grouped in 8 different periods: period 1 - 4 weeks before tx, period 2 - 4 weeks beginning with the day of the 1st injection, period 3 - 4 weeks starting 56 days after beginning of the treatment, period 4 - 4 weeks after the end of period 3, period 5 - 4 weeks after the challenge with GnRH, period 6 - 4 weeks after the end of period 5, period 7 - 4 weeks after the last treatment with Posilac, period 8 - 12 weeks after the end of Posilac treatment.

Results showed that in 9 out 12 bulls Posilac treatment induced a blood increase of IGF-1 secretion. In these 9 bulls the volume of ejaculates increases after the treatment but not the sperm output. Challenge with GnRH, did not increase sperm production significantly in those bulls. This work showed that in bulls older than 5 years of age, BST treatment does not increase sperm cell production. More work need to be done to see if the effect could be observed in younger prepubertal bulls.

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427 (1008)
COMPARATIVE STUDY BETWEEN ISOLATION TECHNIQUES AND PCR FOR MYCOPLASMA AND UREAPLASMA DIVERSUM DETECTION IN PREPUTIAL MUCUS AND SEMEN FROM BULLS USED IN NATURAL BREEDING AND AI CENTERS
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With the objective of improving the mycoplasmosis diagnostic methods, it was accomplished a trial using preputial mucus and fresh semen from bulls in order to reduce the time for report emission and to increase the detection levels, assuming bacterial isolation, lengthy and alive microorganism dependent, as gold standard test. About one hundred seventy five samples of preputial mucus and 143 fresh semen samples were studied. For the PCR technique, standardized and published primers were used. In a previous screening, the MGSO/GPO-1 system was tested for Mollicutes detection. The specific primers for Mycoplasma bovigenitalium and Ureaplasma diversum were also tested. It was observed 45.1% (79/175) of positives for Mycoplasma spp. and 66.5% (115/173) for U. diversum in preputial mucus. Comparatively, the PCR revealed 63.7% (109/171) of positive samples by MGSO/GPO-1 system, 42.6% (72/169) of positive...
samples for M. bovigenitalium and 72.9% (124/170) for U. diversum. Studying the semen samples, 22.5% (32/142) were positive for Mycoplasma spp. and 51.7% (74/143) for U. diversum by isolation. By PCR, it was detected 24.1% (33/137) positive by MGSO/GPO-1 system, 27.4% (34/124) positive samples for M. bovigenitalium and 56.6% (73/129) for U. diversum. McNemar test for Mollicutes presence in preputial mucus samples (p=0.57), showed that MGSO/GPO-1 system could have good value for U. diversum detection, despite the good sensitivity (75.2%) and low specificity (58.9%). The semen analysis showed poor sensitivity 45.2% but good specificity 81.5%. The result of McNemar test showed that MGSO/GPO-1 system could substitute the preliminary isolation for Mycoplasma spp. in semen (p=0.86). The results bring good information for laboratories were the standardization for mycoplasma isolation techniques is difficult. The low sensitivity find in semen was probably due to the several inhibitors that can be present in the reaction. On the other hand, the preputial mucus diagnosis presented a good sensitivity, however, low specificity, that must be increased. The detection method for M. bovigenitalium presents excellent detection levels as much in the preputial mucus as in the semen, justifying the need of the diagnosis in reproducers bulls, especially in semen samples, that should be free of any contamination.

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428 (938)
RISK FACTORS ASSOCIATED TO FIRST BREEDING FAILURE IN DAIRY COWS
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This study investigated the potential risk factors for failure in the conception at the first post-parturition service (CFS). Holsteins cows (n = 427) on a commercial herd of the State of São Paulo had their reproductive, health and nutritional status checked from the pre-partum period to the first service. Occurrence of diseases, body condition scores (BCS) (at the pre and post-parturition), and milk yield and composition after parturition were recorded. A logistic regression model was used to calculate the odds ratio, to determine the risk factors and the associations among the several risks for success or failure in CFS. The principal ranking variables were the lactation number, the season of the year to the first service, the occurrence of diseases in the peripartum, the relationship between the fat and protein percentages of the milk (FPR) and the alterations in BCS. The first lactation animals presented larger chance of success to the first service than the multiparous animals. The inseminated cows in the winter presented, approximately, five times more chances of CFS than the cows inseminates in the summer. Cows that presented health disorders in the peripartum period had smaller conception success than the healthy cows. The animals that presented a better ruminal condition, determined by FPR, mainly during the summer period, had better conception than the animals with reduced FPR in the summer. BCS, appropriate in the different productive phases (dry-off, fresh and first service), demonstrated to be an important tool to identify cows with risk of failure in the CFS.

Key words: Reproduction, Milk components, Body condition score, Dairy cows.
Funding: FAPESP

429 (3209)
COMPARISON OF NEW TECHNIQUES FOR THE DIAGNOSIS OF CHRONIC ENDOMETRITIS IN DAIRY CATTLE
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In veterinary practice the diagnosis of chronic endometritis in dairy cattle is usually based on per-rectal palpation of the genital tract and/or vaginoscopy. Recent studies have shown that subclinical forms of endometritis, not detected by per-rectal palpation, can cause depressed fertility. The objective of this study was to compare three diagnostic methods for the diagnosis of subclinical endometritis on a commercial dairy farm. All cows were examined by per-rectal palpation between day 21 and 27 post partum. Cows without clinical signs of endometritis at per-rectal palpation (i.e. enlarged uterus, purulent discharge) were examined by vaginoscopy (n=110), ultrasonography (n=65), and sampling with the cytobrush technique (n=106). The proportion of cows found affected with signs of endometritis, was 10.9, 42.5 and 58.5 % with vaginoscopy, cytobrush and ultrasonography, respectively. Compared to the cytobrush technique, the sensitivity and specificity of vaginoscopy were 12.3% and 90.2%, respectively. With ultrasonography as “gold standard” they were 7.8% and 96.3%, respectively. Remarkably, the correlation of the results of cytobrush and ultrasonography methods was low. Comparing ultrasonography with the cytobrush technique as “gold standard” the sensitivity and specificity were 57.7% and 40.5%, respectively. A follow up study will evaluate the relationship of both techniques with reproductive performance with a large number of cows.

Studies by Kasimianickam et al. (2001), and Raab et al. (2002) using the cytobrush technique, and Lenz et al. (2003) using ultrasonography have shown a negative impact of subclinical endometritis on reproductive performance. The results of our study have demonstrated the limited diagnostic value of traditional techniques of examination. The ultrasonography and cytobrush techniques both found significantly more cases of endometritis than vaginoscopy. An adequate diagnostic method is the basis for efficient post partum treatment and increased reproductive performance in the current lactation.
430 (1325)
TREATMENT OF REPEAT BREEDING AND CHRONIC ENDOMETRITIS AFFECTED COWS BY USING UTERINE LAVAGE
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Endometritis and repeat breeding syndrome are both the most important causes of infertility in the cows. Uterine lavage alone or in conjunction with hormonal and intrauterine antibiotic therapy has merit in treatment of uterine infections. The aim of this study was to evaluate the effects of this treatment on repeat breeding and chronic endometritis affected cows.

Ninety-four Holstein cows were chosen from a large dairy farm in the suburb of Tehran. Selection was upon the history of chronic endometritis (persistent abnormal vulval discharge) and repeat breeding (more than 3 times inseminations) and clinical examination of the reproductive tract and the ovaries per rectum. Uterine lavage was performed by using a 2-way 18 inch human urinary Foley catheter and both uterus horns were flushed simultaneously by sealing the cervical os with the catheter's balloon. Uterus was flushed by warm (40-45 °C) sterile normal saline (at least two times and 50 ml each time). Eighty-seven percent of flushing fluid was collected in average. Ten million IU penicillin G potassium infused into the uterus after flushing. The cows received 500 mcg cloprostenol the day after flushing for stimulating uterine contractility. Insemination was performed after cows have been detected in estrus.

Sixty-three percent of all treated cows (36% with first, 20% with second and 7% with third insemination or more) became pregnant. Conception rates and services per conception for all cows, chronic endometritis affected cows and repeat breeding cows were “38%; 2.64”, “34%; 2.88” and “53%; 1.87” respectively, after treatment.

The results of this study showed that subclinical endometritis is possibly one of the major causes of repeat breeding syndrome and thus uterine lavage in conjunction with hormonal and intrauterine antibiotic therapy is a useful method for treating chronic endometritis affected and repeat breeding dairy cows.

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431 (746)
URETHRAL EXTENSION AND CASLICK’S SURGERY FOR INFERTILITY TREATMENT IN PNEUMOVAGINA CASES WITH SUNKEN ANUS IN HOLSTEIN COWS
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Rectovaginal injuries including fistula, perineal laceration, pneumovagina and urovagina can result in subsequent infertility and serious economic losses in cows. Pneumovagina results from stretched, ruptured, deformed and horizontal vulva which may introduce fecal material, urine and air into the vagina can cause fertility problem.

In old Holstein cows with pneumovagina problem, especially with sunken anus, probably chronic urovagina and urovaginitis can complicate the infertility problem.

During May 1999 to April 2003 records of 9 Holstein cows (age 7-9 years old) were studied in this report. All cows affected by pneumovagina and none of them had clinical urovagina, but all cows had various degree of sunken anus. Caslick's operation had been done in 9 cows but all of them were infertile for at least 3 artificial inseminations under regular estrous cycle after Caslick's surgery. All cows had no infertility problems according to the veterinary examinations. Urethral extension surgery was done in all cows and Caslick's operation was done again at the same time. All 9 cows were pregnant at the first estrus after surgery.

This report indicates chronic urovaginitis should be considered in old Holstein cows with sunken anus as an infertility problem.

432 (1721)
FACTORS INFLUENCING THE EFFECT OF TREATMENT OF CHRONIC BOVINE ENDOMETRITIS
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It was the aim of the following study to examine the effect of three different treatments of chronic bovine endometritis [intrauterine infusion with 100 ml 4% Lotagen® (n=50) or 20 ml of antibiotics (400 mg Ampicillin + 800 mg Oxacillin; n=49), 5 ml Dinolytic® intramuscularly (n=51)] and no treatment (n=28) on clinical cure and reproductive performance. Furthermore, the following factors that might have an influence on clinical cure and fertility were evaluated: interval between calving and 1st treatment, results of rectal palpation and bacteriology of uterine swabs, presence of a corpus luteum. Cows with chronic endometritis and at least 21 days in milk were randomly subjected to the groups. Overall, clinical cure and clinical cure after 1st treatment was 95.0% and 64.4%, respectively, and did not differ between groups (P>0.05). Reproductive performance based on
fertility parameters and culling rate due to infertility did not differ between groups (P>0.05). With increasing duration of lactation, severity of endometritis decreased and clinical cure after 1st treatment increased (59.5% before Day 42 p.p vs 79.6% after Day 42 p.p.; P<0.05). However, overall fertility was not influenced by the day of 1st treatment. The size of the uterus and the isolation of Arcanobacterium (A.) pyogenes had a negative influence on clinical cure, and the latter one also on reproductive performance. The presence of a corpus luteum did not influence clinical cure and fertility parameters. A multiple regression analysis was performed to test for independent influences, where clinical cure after 1st treatment, first service conception rate and interval from calving to conception (days open) were the dependent variables, and the interval from calving to 1st treatment, size of the uterus, presence of ill smelling vaginal discharge, isolation of A. pyogenes, the treatment and clinical cure after 1st treatment were the independent variables. Clinical cure after 1st treatment was negatively influenced by the presence of A. pyogenes (P<0.001) and no treatment (P<0.05). First service conception rate was negatively influenced by the presence of ill-smelling vaginal discharge (P<0.01) and no treatment (P<0.05). Days open were positively correlated with the interval from calving to 1st treatment and negatively influenced by the presence of ill-smelling vaginal discharge.

433 (5079)
THE EFFECT OF OXYTETRACYCLINE ON PREGNANCY RATE OF “REPEAT BREEDER COWS"
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Repeat breeding syndrome is one of the major problems of cows and leads to high economic losses in dairy herds. It is reported that mild chronic endometritis is one of the most common causes of repeat breeding. It is shown that Oxytetracycline (OTC) has a mild irritating action on the endometrium, results in the mobilization of leucocytes, stimulates uterine motility, and has antimicrobial and flushing action on any mucopurulent materials. The present study was conducted to investigate the effect of intrauterine infusion of OTC (5%), 24h post-insemination in repeat breeder cows with a history of uterine infection after their last parturition.

In this study 116 cows were selected on the basis of repeat breeding syndrome (failed to conceive after 3 services) and a history of uterine infection after their last parturition. Cows with history of pyometra, touchable salpingitis, septic metritis and uterine adhesions were excluded from the study. Then, they were randomly categorized in three groups as follow: Group A) Intrauterine infusion of OTC (25ml), 24h after AI; Group B) Intrauterine infusion of OTC (25ml), 24h after AI plus injection of GnRH (Gonadoreline, 3ml, IM, once) during days 12-14 after AI; Group C) Without any treatment after AI (control group). Group B was considered as a treatment group to clarify that GnRH has not any effect on pregnancy rate in repeat breeder cows with a history of recent uterine infection (to omit the luteal deficiency cause in repeat breeder cows with chronic endometritis).

The pregnancy was diagnosed by rectal palpation during days 45-55 after AI and the results registered as follow: Group A=58.5%, Group B=52% and Group C=32.2%. The pregnancy rate in group C was significantly different from group A and B (P<0.05). The result of this study showed that intrauterine infusion of OTC, 24h after AI leads to improvement of fertility in repeat breeder cows with a history of uterine infection after their last parturition. It shows that they may have a chronic endometritis which had not responded to the past treatments.

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434 (3382)
SEROLOGICAL AND MOLECULAR DETECTION OF BHV-1 AND BBDV IN SEMEN DONOR BULLS IN URUGUAY
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Infectious diseases transmitted by semen are one of the most important factors to take care on the control of reproductive disorders in a herd. Bovine Herpesvirus -1 and Bovine Viral Diarrhea virus (BVDv) are the most prevalent viruses in our country that affect the reproductive tract. The main characteristic of herpesvirus is the capacity to establish latency in the infected animals, so positive bulls become lifelong carriers, with the possibility of virus excretion under stress conditions. In case of BVDv, animals infected during the first trimester could be persistently infected (PI), and bulls can excrete virus continuously, being an important source of infection inside the herd. Artificial insemination (AI) with no controlled semen represents an important risk in transmission of both infections and has to be considered in control of reproductive disorders in cattle. Molecular techniques like polymerase chain reaction (PCR) are more sensitive and less time consuming than virus isolation from semen samples. The objectives of this study were to identify the serological status, to both virus, of bulls at the main Artificial Insemination Centers, and to analyze batches of their frozen semen by PCR. Seventy-five semen and sera samples were analyzed by Elisa test and PCR. Amplification of the extracted DNA from BHV-1 were performed by Nested PCR with two primers pairs from the gl region. In case of BVDv, RNA was amplified by reverse transcription PCR (RT-PCR) using “one-tube"
method with rTth DNA polymerase. Serological results demonstrated high prevalence of both infections: 49.3% of the bulls were antibody positive to BHV-1 and 56% to BVDv. BHV-1 were detected in nine semen samples, and BVDv in two samples from seronegative animals. Virus detected on frozen semen represent a high risk on the dissemination of the infections to susceptible animals. By PCR techniques it is possible to analyze each batch of frozen semen to guarantee the condition of free of virus.

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435 (679)
THE USEFULNESS OF A QUESTIONNAIRE IN THE DIAGNOSIS OF ABORTION OUTBREAKS IN CATTLE HERDS
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The goal of this study was to evaluate the usefulness of a questionnaire for the diagnosis of abortion problems in cattle herds. The questionnaire was created in our clinic based on information in the literature, and it comprised 130 questions. Approximately 10% of them were formulated to address typical features of Swiss dairy farming such as seasonal alpine pasturing and certain aspects of housing like beef calves and dairy cows within the same cowshed. The first author and the herdsmen completed the questionnaires together. Herds with a yearly abortion rate of higher than 10% of pregnant animals were included in the study. A total of 750 cattle farms (745 dairy and 5 cow-calf), distributed throughout Switzerland, was studied from 1986 to 2002. Between 38 and 337 (mean 222) farms could be used for statistical evaluation of each question. The likelihood ratios (LR; or utility of test), P-values and 95% confidence intervals were calculated for each categorical item for positive and negative outcome, and the items were ranked according to the LR. Each farm was allocated either to category “diagnosis” or “no diagnosis” with respect to the abortion problem. Neospora caninum and BVD virus, the most common abortifacients in Switzerland, were of particular interest. The results showed that 41% of all items had a significant (P =0.05) distribution relative to the diagnostic outcome, 23% had a tendency for a significant distribution (0.05 = P-value = 0.2) and 36% of items had no relationship to the outcome. A diagnosis was reached when the following items had a positive answer when evaluated independently: genetic relationship among affected animals (LR=1.51), vaccination program (LR=1.28) and feeding of corn or grass silage (LR=1.23). The significance of the results requires testing for bias, confounding and interactions. The potential usefulness of the most significant items requires further study. More items than expected had a significant distribution relative to the diagnostic outcome, and 36% of the items were diagnostically irrelevant.

436 (2729)
USE OF A PSPB ELISA FOR THE DETECTION OF PREGNANCY IN CATTLE AND BUFFALOES
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Pregnancy Specific Protein B (PSPB) RIA has been used for many years for detection of pregnancy or embryonic mortality in ruminant species. In this abstract the characteristics of an “ELISA sandwich” type assay are described and results compared with those of RIA when used to detect pregnancy in bovine and buffalo cows. Polyclonal antibodies were raised after immunisation with purified bovine PSPB. If present, PSPB binds to the 1st antibody coated on microplates and is captured by a second antibody. Goat immunoglobulin conjugated to peroxidase is added, binds to the 2d antibody and the reaction revealed by TMB. OD is read at 450nm. All incubation steps are made at 20°C. The duration of the assay is about 5 hours.

The sensitivity of ELISA was less than 10 pg/ml. The intra assay CV were respectively of 6% and 4% for bovine serum pools of <0.5 ng/ml and 5 ng/ml. Inter-assay CV's for those pools were 8 and 16%. Intra assay CV's were respectively of 5.7 and 12.7% for pools of buffalo plasma of 0.3 and 3.1 ng/ml and inter assay variation found non significant.

When compared to results of RIA from bovine samples, the full concordance (+RIA/+ELISA or -/-) was 90% (283/314). Five cows had a detectable concentration by ELISA and were found negative by RIA (2%). Other non concordant results were related to low intermediate concentrations (close to 0.5 ng/ml). From buffalo samples, full concordance was 92.1% (117/127). Only one plasma was found negative by RIA and positive by ELISA. In most of other cases, plasmas were classified as positive by RIA and “doubtful” by ELISA. Concentrations measured from the same samples with the two systems throughout pregnancy were very similar by Day 30 of pregnancy (ELISA 1.9 ±0.6 vs RIA 1.7 ±0.3 ng/ml; n=13) but increased sooner and were higher when measured by ELISA than by RIA at later stages of pregnancy. These data show that the ELISA system and the RIA give very similar results when those are expressed qualitatively. The ELISA is sensitive and repeatable enough to be used as a routine test to detect pregnancy in the cow and buffalo.
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In Indian dairy industry, the number of high yielding buffaloes and cattle is increasing especially in Punjab. Most serious problems are reproductive failures including abortions and repeat breeding. Repeat breeding is poorly understood and commonly encountered condition in bovines. Incidence of repeat breeding has been reported to vary from 7.31 to 23.88%. The present investigation communication describes role of abortions and repeat breeding in bovines and it's economic impact. In the present investigation conducted at ten different dairy farms, overall incidence of repeat breeding was 19.61 and 15.63% in cattle and buffaloes, respectively. Maximum incidence recorded at one of the farms was 21.77 and 19.57% for cattle and buffaloes, respectively. Highest incidence of repeat breeding in cattle was in humid hot summer (21.95%) and lowest in winter (18.37%), whereas in buffaloes highest incidence was in dry hot summer (20.62%). Highest incidence of repeat breeding in cattle was in third calvers. Incidence in cattle heifers was 18.70%. In buffaloes, highest incidence of 18.54% was observed in fifth calvers. Sahiwal breed of cows showed highest incidence of 21.67%. Overall incidence of abortions in cattle was 6.74% with highest of 19.14% and lowest of 3.47%. In buffaloes, overall incidence was 5.03%, the highest being 15.15%. In cattle highest incidence of abortions was between 210-240 days of gestation and in buffaloes it was after 240 days. In cattle third calvers had highest abortion rate. Both cattle and buffalo heifers had low incidence.

OSTEOCHONDROSIS IN BEEF SIRES IN SWEDEN
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Most Swedish beef cows and heifers are bred naturally, which makes selection of herd sires a critical decision affecting reproductive performance and profitability. The sustainability of the bull is essential for successful breeding. Bulls with poor reproductive capacity, due to inability to serve, can cause great economic loss. The Swedish insurance company, AGRIA, report that a great number of their insured beef bulls are culled because of lameness. To our knowledge, little is known about hind limb problems in beef cattle, but there are indications that osteochondrosis is a common cause of hind lameness in Swedish beef bulls used for natural service. Osteochondrosis has been studied extensively in horses, dogs, swine and poultry, but less

In conclusion, the present results indicate that lesions compatible with osteochondrosis are common post mortem in Swedish beef sires post mortem, regarding the presence of osteochondrosis.

Right and left hind limb bones from 47 beef sires were examined post mortem to identify lesions in the stifle and tarsal joints. The bulls were slaughtered during or after the breeding season due to lameness (n=27), poor fertility results without signs of lameness (n=16) or because of genetic reasons (n=4). The bulls were of five different breeds, Charolais (n=20), Simmental (n=12), Aberdeen Angus (n=6), Limousine (n=5) and Hereford (n=4), and the median age was 2 years (range 1-7 years). Thirteen of the bulls (28%) were tested at a performance testing station and the rest were non-tested bulls. Forty-two of the bulls (89%) had lesions in at least one joint. Thirty-nine bulls (83%) had lesions in the stifle joints and 17 bulls (36%) had lesions in the tarsal joint. In most of the bulls (86%), the findings were bilateral. The most common site was the lateral ridge of the femur trochlea, followed by the intercondylar eminencia of tibia. Twenty-two of the bulls with clinical signs of lameness (n=27) had joint lesions. All the bulls without clinical signs of lameness (n=20) had lesions in at least one joint. In conclusion, the present results indicate that lesions compatible with osteochondrosis are common post mortem findings in beef sires, regardless of clinical history. The results also indicate that lameness in beef bulls often appear to be caused by osteochondrosis and that a poor fertility outcome can be a result of joint problems.

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STIMULATORY EFFECTS OF ECG AND GNRH IN PERI-PUBERAL HEIFERS WITH DIFFERENT OVARIAN ACTIVITY
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Puberty in beef cattle is an important economic trait. Although several management and pharmacological treatments have been tested, under range conditions, puberty depends mainly on environmental availability of food and not all heifers (two years old) have ovarian activity at the beginning of the breeding season. In order to maximize production, heifers must cycle at the beginning of the breeding season. With the objective to test an ovarian stimulation protocol we used 90 2 years old heifers with in average and body condition scores greater than 3 (ranging from 1 to 5). Heifers were examined by ultrasound and divided into two groups: those with at least one ovarian follicle greater than or equal to 10 mm (F+) and those with follicles smaller than 10 mm or no follicle (F-). Sixty heifers were included in group (F-) and 30 in group (F+). Each of these groups were further subdivided into 3 treatments: group 1 (G1) received 250 IU of (equine chorionic gonadotrophine
i.m. (eCG) on day 0, followed by 250 µg of gonadotrophin releasing hormone, i.m. (GnRH) 48 h latter and progesterin analog F2a analogue (PGF2a 500 µg of cloprostenol, i.m.) on day 12; Group 2 received 250 µg of GnRH on day 2 followed by PGF2a on the day 12 and Group 3 served as a control. All groups were inseminated after estrus detection during 14 days, followed by natural breeding with bulls, during 50 days. Pregnancy was diagnosed by per-rectal palpation 60 days after withdrawal of the bulls. It was expected that eCG might stimulate follicles in both F- and F+ heifers and allow them to respond to GnRH with ovulation and luteolysis only in F+ heifers. The overall pregnancy rate was 44.4%. There was no significant treatment or ovarian activity main effect. However in the control group the ovarian status had a significant effect on the pregnancy rate (70% in group F+ and 5% in group F-). These results indicate that in Groups 1 and 2 eCG and GnRH had a significant stimulatory effect in heifers without ovarian activity (F-) whereas in Group 3 (control group) F- heifers had a delayed occurrence of puberty in comparison with the group F+ resulting in a lower pregnancy rate at the end of the breeding season.

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440 (1300)
IMPROVEMENT OF FERTILITY IN REPEAT BREEDER COW BY PGF2ALPHA ANALogue

ADMINISTRATION
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The aim of this study is to assess whether the injection of a PgF2a analogue (d-cloprostenol, Dalmazin R - Fatro, Italy) in repeat breeder cows at the time of artificial insemination (AI) improved the fertility of treated animals and increased their conception rate. On the basis of recent research (Kobayashi and Miyamoto, 2000), we believe that PgF2a may positively affect the CL formation and activity. To confirm our hypothesis, two groups of healthy and normally cycling cows were treated with indomethacin, a selective inhibitor of cyclo-oxygenase and, therefore, of PgF2a, with single or multiple injections, respectively. The study was carried out on 120 repeat breeder (group A and D) and 20 (group B and C) normally cycling Friesian cows. The cows were divided into 4 groups: group A (PgF2a) comprising 60 cows, group B (Indomethacin, single injection) of 10 animals, group C (Indomethacin, multiple injection) of 10 animals, and group D (control) consisting of 60 cows. At AI, the animals of group A received an intramuscular injection of 2 ml of a PgF2a analogue (75 g/ml of d-cloprostenol - Dalmazin R, Fatro, Italy). Group B (indomethacin, single injection) received intramuscularly an injection of 8 ml of indomethacin (25 mg/ml of Indomethacin meglumine - LiometacinR, Promedica - Italy) and group C (indomethacin, multiple injection) received intramuscular injections of 8 ml of indomethacin (25 mg/ml of Indomethacin meglumine - LiometacinR, Promedica - Italy) for 5 consecutive days following AI. Group D (control) received 2 ml of physiological saline solution intramuscularly at the time of AI. Statistical analysis of the results obtained was performed by chi-square test and P<0.05 was considered to be statistically significant. d-cloprostenol determines an increase of plasma progesterone concentrations and corpus luteum diameter thus improving conception rates (66% in the treated group versus 18% control group with P<0.05), while indomethacin inhibits the development and function of the corpus luteum, mostly in the group B, suggesting the presence of a luteotropic prostaglandin activity, when d-cloprostenol is used at the time of AI.

441 (2821)
THE INTERVAL BETWEEN ESTRUS, THE LH SURGE AND OVULATION IN HOLSTEIN CATTLE; A RE-APPRAISAL
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Fertility of Holstein cows has decreased for years and recently, this trend also was observed in heifers. This may be due to a different chronology of events leading to ovulation for those animals bred nowadays when compared 10-15 years ago. Therefore, 2 experiments (exp 1 and 2) were designed to investigate the relationships among estrus behavior, time of occurrence of the LH surge and time of ovulation in Holstein cows and heifers.

In exp 1, 12 heifers had their estrus synchronized twice 3 weeks apart using the Crestar method. After implant removal, animals were observed every 4 hours for onset of estrus (standing when being mounted by a herd mate), occurrence of ovulation (ultrasonography) and blood sampled to follow LH patterns (RIA). In both replicates, all heifers displayed estrus, had an LH surge and ovulated. None of the measured parameters differed between replicates; therefore, results were pooled. The intervals between estrus and ovulation, estrus and the LH peak, and between the LH peak and ovulation were respectively 38.5 h ± 3.0, 9.1 ± 2.0 and 29.4 h ± 1.5 (mean ± sem). Linear regression analyses showed that the variation in the interval from estrus to LH peak explained 80.6% of the variation in the interval from estrus to ovulation.

The variation in the interval between estrus and the LH peak was evaluated in exp 2 by using 12 pubertal Holstein heifers and 35 Holstein cows. The same protocol was applied (but time of ovulation not studied) and progesterone (P4) was measured by RIA the day of estrus (D0), D7 and D14. All heifers were observed in
estrus and had an LH surge. They ovulated and had a functional CL as evidenced by P4 levels. Among cows, 30/35 displayed estrus and 33/35 had an LH surge. Every cow with an LH surge had a functional CL. Statistical analysis of differences between heifers and cows including data of exp 1 and 2 showed that the duration of the interval between the beginning of estrus and the LH peak was longer in heifers than in cows (4.15 h vs -1.0h; P <0.002) but the variation for this interval was higher in cows than in heifers (sem= 1.2 h vs 0.8 h; P=0.01).

The long interval between the onset of estrus and ovulation (exp 1) and the high variation of intervals between estrus and LH peak (exp 1 and 2), especially in cows, may be a source of low fertility and should be considered when analysing reproductive disorders.

442 (3381)
POSTPARTUM CLINICAL ENDOMETRITIS IN NORMAL AND REPEAT BREEDER DAIRY COWS AND ITS IMPACT ON REPRODUCTIVE PERFORMANCE UNDER THE FREE GRASSING PRODUCTION SYSTEM OF URUGUAY
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A prospective case-control study was performed to evaluate the relationship between repeat breeder condition (RB) and postpartum endometritis and posterior reproductive performance. The study was conducted in six dairy farms, with 228 multiparous cows that calved in fall months of 2003. Case group (RBg) had 114 cows with history of more than three services to become pregnant. Control group (Ng) had 114 cows with the same number of parity than case cows, parturition date within fifteen days range and required less than four services in their previous pregnancy. General and reproductive postpartum examination, including, transrectal palpation and vaginoscopy, were performed once a month in both groups, from April to October 2003. A diagnosis of postpartum endometritis was established when a mucopurulent or purulent discharge was observed by vaginoscopy. No treatment was performed if endometritis was present less than 90 days postpartum. All data of cows were recorded, including culling number and reasons. New pregnancies were determined by transrectal palpation at day 35-50 postservice. Associations of reproductive conditions in both groups were evaluated by Chi-Square Test. Clinical postpartum endometritis was found in 48 cows (21%). The condition was similar in booth groups: 22 cows in Ng and 26 in RBg. (Chi-Square = 0.61; ns). After six months of the study, 20 cows were culled (8.8%): 8 of the Ng and 12 of the RBg. No association was observed related with the RB previous condition: Chi-Square = 1.03; ns). At the end of October, pregnancy was confirmed in 158 cows: RBg = 74 and Ng = 84, representing 76% of the remaining population. No differences were detected between groups (Chi-Square = 1.05; ns). The results of this study indicated that repeat-breeder condition is not necessary related with poor reproductive performance. If we accept the traditional criteria for RB probably we will cul cow that are only “statistically RB”. These cows must be differentiated from the “real RB”, with a strong history of more than four, six or even nine services to become pregnant in subsequent pregnancies.

Funding: INIA LIA 024

443 (2945)
FOLLICULAR DYNAMICS AFTER PROGESTIN TREATMENT IN BEEF CATTLE.
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Progestins and Estradiol Benzoate (EB) have been used in the induction and synchronization of estrus in cattle with the goal of dominant follicle regression and the liberation of PGF2α for CL regression. The recruitment, dominance and development of dominant follicles depend on hormones such as FSH, free IGF and LH acting in the theca and granulosa cells. Depending on the state of the estrus cycle and the concentration of progesterone, estradiol inhibits the transcription FSH, and influences the frequency of GnRH pulses, consequently influencing LH. Due to the different hormonal actions in the regulation and development of the dominant follicle, this experiment was developed to determine the effect of a progestins withdrawal (pessary vaginal with 250 mg medroxi acetate progesterone - MAP) and EB on the follicular dynamics of beef cattle of different reproductive status. Thirty cows were divided into 3 groups by reproductive status. Group 1: postpartum anestrous multiparous cows, weaned around 60 days postpartum (without CL). Group 2: Multiparous cycling cows (with CL). Group 3: anestrous heifers (without CL). The animals were selected using gynecological examinations. They received MAP for seven days and 5 mg EB im at the time of pessary insertion. After withdrawal of the pessaries, ultrasound scanning was performed daily for 4 days to evaluate follicular dynamics. The difference between the diameters the two largest follicles (deviation) was greater (P<0.001) in cycling cows (4.7 mm) than anestrous cows (3.2 mm) and heifers (2.4 mm). It became possible to detect dominance followed by the regression of the second largest follicle characterizing deviation. It may be concluded that the protocol of MAP plus EB can be used not only to synchronize estrus but to induce ovarian activity in anestrous cattle since treatment caused a new follicular wave with recruitment, dominance.
and deviation after withdrawal of the intravaginal pessaries.

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444 (1874)
ISOLATION OF HAEMOPHILUS SOMNUS FROM PNEUMONIC LESIONS OF SLAUGHTERED CATTLE
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H. somnus is a gram-negative coccobacillus that can infect many body systems of cattle including respiratory, nervous and urogenital systems. H. somnus is being isolated with increasing frequency from animals experiencing pneumonia and pleuritis.

So far, there was not any report on H. somnus respiratory infection from Iran. A study was performed to isolate the bacterium from pneumonic lungs. Fifty-two samples of pneumonic lungs were collected from slaughterhouses in suburbs of Tehran. The samples were cultured in relevant media by standard method. Antibiograms were done on H. somnus positive cultures to find out which available antibacterial drugs could do well against the bacterium in vitro.

The results indicated that H. somnus was isolated from 4 of 52 (7.69%) samples. This frequency seems to be lower than other countries such as Japan (40%). Other bacteria such as E. coli, P. multocida, M. haemolytica B. sereus were also isolated from pneumonic lesions. Antibiogram results showed H. somnus was sensitive to Ceftriaxone (Exenel®), Enroflaxacin, Gentamycin, Oxytetracycline and Tylosin respectively, and was resistant to Trimethoprim and Streptomycin.

Funding: Tehran University

445 (1363)
INTERACTIONS BETWEEN TOXIC AND NUTRITIONAL ESSENTIAL ELEMENTS IN THE TISSUES OF CATTLE FROM NW SPAIN
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Since the toxicity of one metal or metalloid can be dramatically modulated by the interaction with other toxic or essential metals, studies addressing the chemical interactions between trace elements are increasingly important. In this study correlations between the main toxic (As, Cd, Hg and Pb) and nutritional essential (Ca, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements were evaluated in the tissues (liver, kidney and muscle) of 120 cattle from NW Spain, using Spearman rank correlation analysis based on analytical data obtained by ICP-AES. Although accumulation of toxic elements in cattle in this study is very low and trace essential metals are generally within the adequate ranges, there were significant associations between toxic and essential metals. Cd was positively correlated with most of the essential metals in the kidney, and with Ca, Zn and Cu in the liver. Pb was significantly correlated with Co and Cu in the liver. A large number of significant associations between essential metals were found in the different tissues, these correlations being very strong between Ca, Cu, Fe, Mn, Mo and Zn in the kidney. Co was moderately correlated with most of the essential metals in the liver.

In general, interactions between trace elements in this study were similar to those found in polluted areas or in experimental studies in animals receiving diets containing high levels of toxic metals or inadequate levels of nutritional essential elements. These interactions probably indicate that mineral balance in the body is regulated by important homeostatic mechanisms in which toxic elements compete with the essential metals, even at low levels of metal exposure. The knowledge of these correlations may be essential to understand the kinetic interactions of metals and their implications in the trace metal metabolism.

446 (3070)
TRANSPORT OF ANIMALS IN THE E.U. IN THE LIGHT OF A RECENT EUROPEAN COMMISSION PROPOSAL
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The road transport industry is subject to an ongoing series of Community regulations which aim to establish specific procedures which require qualified personnels and strengthen checking system. Directive 91/628/EEC, amended by Directive 95/29/EC, constitutes the current Community legislative framework. Council Regulation (EC) no. 1255/97 and Council Regulation (EC) no 411/98 were adopted subsequently to provide more detailed rules. The proposal from the Commission regarding this field repeals all existing Community laws in relation to the protection of animals during transport. It also incorporates the requirements of the Commission proposal for a Council Regulation amending Regulation (EC) no 411/98 as regards ventilation in road vehicles carrying livestock on long journeys.

The process of revision has been developed following the recommendations of the Scientific Committee on Animal Welfare and by close scrutiny of the economic impact of the proposed measures. The aim of the Commission proposal is to guarantee that the high standards recommended by the Scientific Committee are
fulfilled via the creation of proper legislative instruments allowing competent authorities to perform better control and enforcement. Transport of animals is part of the process of animal production. Their movement is necessary to adjust the unequal distribution of resources and demand and link between animal welfare, food safety and animal health issues is generally recognised.

Taking into account the fundamental modifications in the proposal which derive from the experience acquired by Member States and from the review of the latest scientific data, the recent proposal from the European Commission is the most appropriate means of achieving the desired objective.

447 (3295)
EVALUATION OF ENTEROBACTERIACEA BETA-LACTAMASES PRODUCTION, MULTIPLE RESISTANCE AND THE SENSITIVITY PATTERN OF ISOLATES FROM CLINICAL AND SUBCLINICAL BOVINE MASTITIS
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The pathogens of mastitis can be divided into two groups, the contagious and environmental pathogens. Among the main etiologic agents of bovine mastitis are the microorganisms of the Enterobacteriaceae Family. The purpose of this paper was the “in vitro” evaluation of the sensitivity pattern showed by these microorganisms isolated from clinical and subclinical bovine mastitis cases tested against some antimicrobials used in the treatment and control of this disease. It was evaluated the “in vitro” sensitivity of 154 Escherichia coli strains isolated from bovine mastitis and the detection of b-lactamase production by these microorganisms. All the “in vitro” sensitivity tests were performed by Kirby & Bauer diffusion method in Mueller Hinton agar, it was used sixteen different antimicrobials and the betalactamase production was performed by the iodometric method and the extended-spectrum beta-lactamases detection. During the period of 1996 to 1998 a total of 13804 lactating cows from 129 dairy herds were studied and 5532 lactating cows of 50 dairy herds were examined during the period 1999 to 2001 The studied Escherichia coli strains showed the highest level of resistance to ampicillin, cephalotin, tetracyclin. In relation to the beta-lactamase production it was verified that it was produced by 98.05% by the iodometric method and 50% of the tested strains showed positive results to the extended-spectrum beta-lactamases. It was also verified the occurrence of multiple resistance among the 154 E. coli isolates, from these 18.95% showed resistance to at least five different antimicrobials, 33.33% were resistant to at least three antimicrobials, and one of them were resistant to ten out the sixteen antimicrobials evaluated. The multiple resistance is very important considering the Human Health as well as the bovine and deserves more attention by the national researchers. Attention must also be drawn to the plasmidial transference of resistance to the microbiota of the milk consumers.

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448 (1321)
CHARACTERISATION OF SALMONELLA CONTAMINATION IN FRENCH BOVINE DAIRY HERDS
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Since several decades, Salmonella is the first cause of food-borne outbreaks in France. For instance, in 2001, Salmonella represented 64% of the cases for which the pathogen was identified. Eggs and eggs based products, delicatessen, milk and milk products were the most implicated foods in Salmonella outbreaks (Haeghebaert et al, 2002).

In order to get information on the circulation of Salmonella strains in bovine dairy herds, two studies were conducted in our lab with characterization of isolates with molecular markers using PCR: RAPD, ERIC-PCR, PCR-ribotyping and IS200-PCR (Millemann et al, 2000).

First, we studied 128 S. bredeney isolates recovered over a three-years-period from a well-controlled farm which comprised two distinct buildings: one for the heifers, one for the cows. Molecular markers revealed the genomic diversity of the isolates, but also the presence of two major clones, harboured by animals in both buildings. By contrast, the characterization revealed the presence of distinct clones specific to either heifers or cows. Moreover, we established that individuals were harbouring successively different clones in their gut as dominant Salmonella strains.

Second, within the frame of a study on risk factors of Salmonella contamination of bulk milk, a study of the diffusion of salmonellae in bovine dairy herds was conducted in order to assess clonal ties among Salmonella isolates as well as routes of contamination within a herd and between herds. 129 Salmonella strains belonging to 6 different serovars were isolated from bovine dairy herds and characterised. This led to the definition of 15 different PCR-types (by the combination of the different patterns) and allowed to get a dendrogram showing genetic similarities between strains. This work allowed us to confirm the hypothesis of the contamination of each dairy herd by a single clone. By contrast, no relation could be found between isolates found even in closely related farms.

In conclusion, those studies allow us to precisely analyse modes/ways of contamination by Salmonella at the farm level as well as at the farming region level. This can help for instance to adapt on farm control measures.
Further studies are needed to get data on the extension of the prevalence of this contamination in farms experiencing or not clinical cases of salmonellosis.

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449 (1432)
DIVERSITY AND RULES: WHICH RULES FOR A SUSTAINABLE MANAGEMENT OF PASTORALES RESOURCES IN VINA (CAMEROON)?
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The functioning of some livestock systems in sub-Saharan Africa tends to give reason to the partisans of the "tragedy of commons" theory. In response, some experts and policy makers recommend settling as an imperative for development and the protection on the environment. Results of research activities carried out in Vina, one of the main cattle breeding zone in Cameroon, suggest that those reasoning should be looked over again. The zoning of Vina according to livestock activities shows that there is a tight link between the area of intensification of livestock farming system and the gradient of resources' damage. A study of the cattle's market channel reveals that the increase of cattle fattening and milk production units around Ngaoundere is due to the strategic location of this town which is at the same time an important urban center and also the collection and boarding point of cattle in destination to "foreign markets". Thus intensification of productions contributes to the damage of pasture land. Paradoxically, pastures of transhumance zones are less damaged and show some resilience. This resilience could be due to the seasonal pattern of grazing practiced there. However, free access pastures which are very often used all around the year are more damaged. Cattle breeders don't go beyond this statement yet, and very often prefer emigration than searching for adequate solutions necessary to keep up and restore those pasture land. In a mid run, the continuation of cattle breeding activities in Vina will depend on the capacity of policy makers and all the stakeholder involved in the cattle breeding and market channel to draw up and apply rules which aimed at a sustainable management of resources. For transhuman, the tasks will be to define collective rules for access and use of resources. However, for sedentary semi-intensive cattle breeders, the problem is to define technical norms both animal charging and farming infrastructure level. Beside these rules, it is also important to improve roads and access to markets; this will help to alleviate pressure on resources through an increased renewal rate of herds and the relocation of some activities.

Keys words: Livestock farming systems, sustainable management, zoning, market channel study, rules, Vina (Cameroon)

Funding: GESEP

450 (2168)
A SURVEY OF THE PREVALENCE OF BHV-1 INFECTION IN AHWAZ DISTRICT AT THE SOUTH OF IRAN
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Bovine herpes virus-1 (BHV-1) is one of the most important bovine viruses which cause a variety of clinical syndromes, including infectious bovine rhinotracheitis, infectious pustular vulvovaginitis, abortion, conjunctivitis and a systemic infection in calves. Cattle are the principal reservoir and usual source of infection. The virus is perpetuated in bovine population, largely by direct contact. The aim of the present study was to determine the seroprevalence of BHV-1 infection in cattle population of Ahwaz district at the south of Iran. For this purpose, a total of 521 sera from Holstein (114 sera), hybrid (271 sera) and native (136 sera) cattle were collected and tested by a commercial ELISA kit. The result showed the seropositivity of 38.6, 31 and 34.5% in Holstein, hybrid and native cattle, respectively. The result indicates a moderate prevalence of the BHV-1 infection in the region.

Funding: Shahid Chamran University

451 (3457)
ANTIMICROBIAL SUSCEPTIBILITY AND GENETIC RELATEDNESS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM DAIRY HERDS IN NORTH CAROLINA AND VIRGINIA
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Mastitis caused by Staphylococcus aureus is particularly difficult to control and treat. Treatment of established infections with antibiotics, either in dry or lactating cows, is much less effective than desired. Our objective was to investigate antimicrobial resistance of bovine mastitis-causing S. aureus isolates and to determine if resistance relates to specific S. aureus type as defined by pulsed-field gel electrophoresis (PFGE) band patterns. Minimum inhibitory concentrations (MIC's) of 14 antibiotics or combinations of antibiotics (amoxicillin-clavulanic acid, cephalothin, clindamycin, erythromycin, gentamicin, oxacillin, penicillin, tetracycline,
trimethoprim/sulfamethoxazole, vancomycin, ceftiofur, enrofloxacin, penicillin/novobiocin, and pirlimycin) were determined for S. aureus isolated from milk samples collected periodically from February, 1988 to September, 2001. Pulsed-field gel electrophoresis was also performed on each isolate. Electrophoretic band patterns (EP's) determined by PFGE were visually analyzed, and a dendrogram was created using a gel analysis program. EP's clustered above a similarity level of 80% on the dendrogram were considered to be genetically similar. Each cluster of related EP's was considered a separate type, and was given a type number. No more than 1 isolate per cow was used, except where more than 1 EP was found. Only those types with 5 or more isolates were included. A total of 357 isolates, representing 7 types of S. aureus from 24 farms, were analyzed. Isolates were from dairy herds in North Carolina (351/357) and Virginia (6/357).

Only 49 S. aureus isolates showed resistance to any of the 14 antibiotics tested, 37 to penicillin only, 1 to erythromycin alone, 2 to tetracycline alone, and 9 to both penicillin and erythromycin. Resistance was found in types 1, 4, 5, and 7 in 32/49, 4/5, 2/11, and 11/11 isolates, respectively. The resistant types were found in milk samples from only 3 of the 24 farms.

In summary, the majority of bovine mastitis-causing S. aureus studied showed little resistance to any of the antibiotics tested. However, those S. aureus isolates that demonstrated resistance appeared to be restricted to specific types as defined by PFGE electrophoretic band patterns.

Funding: NC Dairy Foundation, US FDA

452 (2107)
HYGIENE IN A PORTABLE MILKING MACHINE, USING AUTOMATIC WASHING VS HAND WASHING
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The objective was the quantification of colony forming units per millilitre (cfu/ml) of aerobic mesophylls (AM) and total coliforms (TC) in Petri dish, from the interior of a milking unit after having been washed by CIP (Cleaning in Place) compared to the hand washing of the portable milking equipment. The design of the experiment included group G1 in which the milking machine was washed with the CIP equipment; in G2 the washing was done by hand. Ten samples we taken from the municipal water source to determine the cfu/ml of AM and TC (control). The mineral content of water was determined by colorimetry, and from the result, the concentration of detergent, necessary to wash the milking machines was established. The 2nd sample of water for the bacteriological study; including 20 repetitions in each experimental group, with one week intervals between samplings. The same procedure was performed upon G2 with washing done by hand. The analysis of the samples was performed following the methods described by the Mexican Official Standards NOM-110-SSA1-1994, NOM-113-SSA1-1994. With the result of the samples of water, positive to growth of cfu/ml of AM and TC (positive sample is the one with growth of at least 1 cfu/ml), the difference between the two proportions was analysed using a hypothesis test ("Z" Test) to contrast that difference at a significance level of 0.05. The municipal water came out negative to AM and TC. When comparing the milking machines, after performing the two washing methods, and comparing the proportions of positive samples, a significant difference was found for AM in G1 65% and G2 95% (P<0.05); for CT in G1 the proportion was 30% and G2 80% (P< 0.05). The positive proportions including AM and TC, in the wash water samples, were significantly different: G1 35% and G2 70% (P<0.01). After the last rinse of re-circulated water, including both AM and TC, for G1 the result was 47.5% and for G2 87.5% with a significant difference (P<0.01). The conclusion is that, from the sanitary point of view, there is a significant disadvantage when the machine is washed by hand when compared to the CIP wash.

Funding: Research

453 (2413)
EVALUATION OF THERAPEUTIC EFFICACY AND DISPOSITION PATTERN OF ERYTHROMYCIN IN MASTITIS IN DAIRY COWS
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The purpose of study was to determine the in vivo therapeutic efficacy, serum bioequivalence and milk drug residues of erythromycin following its intramammary infusion in mastitic quarters of dairy cows. The therapy trial involved 34 specific mastitis quarters (culturally positive and SCC > 500 000 cells/ml, IDF criteria) of HF x Sahiwal cows in their early lactation. The diseased quarters were infused with erythromycin* 300 mg/quarter, q 12 h x 5 times. A quarter was considered cured when culturally negative and had SCC < 500 000 cells/ml on day 21-28 post-last infusion (PLI). The effect of therapy on biochemical composition of milk was also evaluated. The drug could cure 73.53% of mastitic quarters; in specific 70.59, 83.33, and 72.73% of Staphylococcus aureus, coagulase negative staphylococci and streptococci infections, respectively. A significant improvement was seen in the milk composition. The pre- and post-therapy values (Mean ± SD) for the cured quarters were electrical conductivity (5.95 ± 0.45 Vs 5.35 ± 0.43 mS/cm), lactose (3.88 ± 0.51 Vs 3.88 ± 0.51), total protein (2.71 ± 0.45 Vs 2.82 ± 0.34), SNF (7.84 ± 0.37 Vs 8.22 ± 0.28%), and pH (6.81 ± 0.08 Vs 6.74 ± 0.08). The assay of drug residues (Microbial assay, test sensitivity 0.05 µg/ml) in 13 cows treated for specific mastitis revealed passing of drug in milk of treated quarters at levels of 15.56 ± 1.35, 0.46
± 0.12 and 0.05 ± 0.00 µg/ml at 1st, 2nd and 3rd milking PLI, respectively. The drug crossed to untreated quarters up to 1st milking in 7/8 cows treated for two or more quarters while in 1/5 cows treated for one quarter. In no case drug was detected beyond 3rd milking (36 h), and only a fraction of infused dose (4.12 to 10.52%) excreted in milk. Study on disposition pattern showed that following intramammary infusion drug appeared in blood at 15 min and maintained for more than 12 hours. The pharmacokinetic analysis of plasma levels-time curve revealed area under curve, mean residual time, elimination half-life and apparent volume of distribution as 12.84 ± 0.85 µg/ml.h, 23.11 ± 0.95 h, 16.02 ± 0.66 h and 1.17 ± 0.07 L/kg, respectively. The results indicated rapid and extensive penetration of erythromycin in body fluids and tissues, and its slow clearance from the body of animal. It is concluded that use of drug in therapy of mastitis may be beneficial owing to its high therapeutic efficacy, good tissue distribution and short milk withdrawal period.

*Bovimast from Indo-Biocare Pvt. Ltd., Vadodara, India

Funding: State Government

454 (5035)

AN EVALUATION OF THE PORTASC® TEST AS AN ESTIMATE OF MILK SOMATIC CELL COUNT

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Udder health management programs often rely on indirect measures to detect subclinical intramammary infections (IMI). There is an increasing need for a rapid, accurate cowside test to estimate somatic cell count (SCC), as a preliminary screening test for IMI. An investigation was conducted to determine the epidemiological test characteristics of the PortaSC™ Test (PortaScience Inc, Moorestown, NJ).

Approximately 600 aseptically collected quarter milk samples were used to determine the test characteristics of the PortaSC™. All samples were subjected to milk bacteriological culture, and the SCC was determined using a Bentley 300 Somacount machine at the University of Guelph Mastitis Research Laboratory. For the test characteristics, a level of >200 000 cells/ml was used as a threshold value, above which a sample was considered to be disease positive and below which was considered disease negative. Statistical evaluation of the results from the PortaSC™ test and the ln(SCC) showed a highly significant association between these tests, with a p-value of <0.00001 and a Spearman Rank co-efficient (R^2) of 0.6281. The correlation between ln(SCC) and the presence of bacterial pathogens was also evaluated. Despite a positive association between these tests, the correlation coefficient was not significant. The PortaSC™ test had a sensitivity of 76% and a specificity of 94%, when compared to the gold standard SCC level. The PortaSC™ test was found to have a predictive value of a negative test of 93%. The product usability and ease of interpretation was also assessed.

In summary, the PortaSC™ test is a fast and accurate method of SCC estimation that can easily be used on farm. However, it should not be used as a direct indicator of IMI. The high predictive value for a negative test suggests considerable potential for its future use by dairy producers in their dry cow treatment decision-making protocol.

455 (1244)

EFFICACY OF PIRLIMICIN FOR S. AUREUS IMI

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A new antibiotic for the therapy of mammary infection (IMI) caused by S. aureus has been recently introduced (2002) in Europe: the Pirlonimicin. The registered therapeutic protocol prescribes the intramammary administer of 50 mg q 24 h for 8 days in the infected quarter. The aim of this study has been to value effectiveness of Pirlonimicin (Pirsue®, Pharmacia Animal Health) in cows with different mammary conditions (Score 1= subclinical infection with no altered mammary parenchyma; Scored 2= milk alteration with light atrophy or sclerosis), with different number of calvings (primiparous and pluriparous) and with different therapeutic protocols (4 and 6 days used).

We have considered 84 infected cows (2 samples, NMC method) in 14 dairy farms and 183 infected quarter. Thirteen cows (33 quarters) have been treated for 8 days, 21 cows (33 quarters) for 4 days and 50 cows (117 quarters) for 6 days. The presence of S. aureus and the reduction of somatic cells count have been verified and planktonic colonies and the presence of bacterial pathogens was also evaluated. Despite a positive association between these tests, the correlation coefficient was not significant. The PortaSC™ test had a sensitivity of 76% and a specificity of 94%, when compared to the gold standard SCC level. The PortaSC™ test was found to have a predictive value of a negative test of 93%. The product usability and ease of interpretation was also assessed.

In summary, the PortaSC™ test is a fast and accurate method of SCC estimation that can easily be used on farm. However, it should not be used as a direct indicator of IMI. The high predictive value for a negative test suggests considerable potential for its future use by dairy producers in their dry cow treatment decision-making protocol.
(67%) has been achieved in pluriparous cows and/or in animals with lesions in udder parenchyma. Therefore, this study demonstrated either the effectiveness of pirlimicin or the importance to examine the cows before deciding whether to treat them or not and the length of S.aureus IMI therapy.

456 (1334)
HIGH PREVALENCE OF SUBCLINICAL AND CLINICAL MASTITIS CAUSED BY ENVIRONMENTAL AND MINOR PATHOGENS IN A LARGE DAIRY HERD IN IRAN
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Environmental and minor pathogens have increasing significance especially in situations where major contagious pathogens have been controlled by post-milking teat dipping and dry-cow therapy. Coagulase-negative staphylococci (CNS) are the most prevalent bacteria in herds isolated by currently recommended control measures and Corynebacterium bovis (C. bovis) was identified as the cause of up to 20% of all cases of clinical mastitis in some herds. The study was performed to determine the etiology and prevalence of mastitis in a large dairy herd in Tehran province where a high incidence of clinical mastitis and the presence of Strep ag in bulk tank was noted. The herd had not used a proper mastitis control routine, particularly concerning the control of environmental mastitis. For the high incidence of clinical mastitis, a high proportion of cows were usually out of the tank. A few months before the beginning of the study, the herd had experienced an outbreak of fatty liver syndrome with 15 clinically affected dairy cows. Composite milk samples were aseptically collected from all lactating cows (n=852) for bacteriologic culture. Microbiological procedures were conducted in accordance with National Mastitis Council (NMC) standards. Culture results revealed 461 (54.11%) cows were subclinically and 97 (11.38%) were clinically infected with environmental and contagious pathogens with an overall point prevalence rate of 65.49%. Coryn. bovis was isolated from 145 (31.45%) subclinical and 26 (26.8%) clinical cases, coliforms from 128 (27.76%) and 27 (27.83%), environmental streptococci from 91 (19.74%), and 10 (10.3%), CNS from 84 (18.22%) and 3 (3.09%), Strep. ag. from 32 (6.94%) and 7 (7.21%) and Staph. aureus from 18 (3.9%) and 4 (4.12%) clinical and subclinical, respectively. Results indicated a high prevalence of subclinical and clinical mastitis caused by environmental coliforms and streptococci and by minor pathogens (Coryn bovis and CNS). These results suggest that an effective mastitis control program should consider other aspects of dairy enterprise including nutritional and metabolic programs. In addition, although C. bovis is historically considered as a minor mastitis pathogen, these results add weight to some reports suggesting this bacterium as a major pathogen of subclinical and clinical importance. If C. bovis appears to be a major pathogen, further studies on more effective control of this organism are needed.
Funding: University of Tehran

457 (3170)
NUCLEAR FACTOR KAPPAB INHIBITION IN BOVINE MAMMARY EPITHELIAL CELLS REDUCES INTRACELLULAR INFECTION BY STAPHYLOCOCCUS AUREUS
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Staphylococcus aureus (S. aureus) is the most common cause of bovine mastitis, which is a disease responsible for important economic losses in dairy production. Despite the progress in antimicrobial therapy, the treatment and prevention of S. aureus infection remain problematic. S. aureus infection often resists antibiotic therapy and therefore often tends to become chronic. Recently, it was reported that chronic mastitis is characterized by a persistent activation of the nuclear factor kappaB (NF-kB) (Boulanger et al., 2003). The objective of the present study was to determine whether the perpetual activation of NF-kB plays a role in the intracellular infection of S. aureus. For this study, two pharmacological NF-kB inhibitors, 15-dPGJ2 and BAY, were used to treat bovine mammary epithelial cells (MAC-T cells). In MAC-T cells, NF-kB is constitutively activated at a lower level. Firstly, different concentrations of 15-dPGJ2 and BAY were tested to determine the minimal NF-kB inhibitory concentration. It was demonstrated that each drug inhibited NF-kB at 20 µM. Secondly, toxicity of each inhibitor used at minimal inhibitory concentration was evaluated by apoptosis assay. The observed apoptosis rate was not significant. Finally, the effect of NF-kB inhibition on S. aureus infection was analysed. Confluent cells were first treated with 15-dPGJ2 or BAY at the minimal inhibitory concentration. After 1h, the cells were inoculated with a suspension of bacteria. After incubation of 1h, the cells were washed once with PBS and medium supplemented with gentamicin was added. The cells were incubated for an additional hour with the gentamicin to kill extracellular bacteria. The cells were washed 3 times with sterile PBS, trypsinized, and then lysed by addition of sterile deionised water. The cell lysates were diluted and plated on Nutrient Agar to quantify intracellular staphylococci. It was observed that treatment of MAC-T cells with 15-dPGJ2 or BAY significantly reduced the penetration of S. aureus. A concentration of 20 µM of 15-dPGJ2 and BAY induced a reduction of 70% and 56% of intracellular bacteria, respectively. In conclusion, we postulate 1) that basal NK-fB activity is required for penetration of S. aureus into mammary epithelial cells, and 2) that pharmacological NF-kB inhibitors could be used to reduce the intracellular infection of S. aureus.
Funding: Ministère de la Santé Publique

458 (1986)
INVESTIGATIONS ON PENETHAMATE HYDRIODIDE (MAMYZIN®) USE IN HEIFERS PRECALVING ON THE INCIDENCE OF MASTITIS WITHIN 7 DAYS POST PARTUM

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Introduction: In the spring of 2002 a prospective cohort study was carried out on the systemic treatment of penethamate hydriodide on heifers pre-calving within a commercial dairy herd with approximately 230 Holsteins in Central Southland, New Zealand.

Objective: This field trial investigated the effect of an intramuscular treatment with penethamate hydriodide prior to calving in heifers on the subsequent incidence of mastitis within 7 days post calving.

Materials and Methods: 89 heifers calved in this period; 35 were untreated (controls); 54 were treated with a single intramuscular treatment of 15 mg penethamate hydriodide per kg body weight (Mamyzin®, Boehringer Ingelheim GmbH) at 7 days before expected calving date. The mastitis incidence at and 7 days after calving and the number of culled animals for mastitis reasons were evaluated. The statistical methods used for evaluation were t-tests with a significance level of p=0.05.

Results: Heifers calved within 3-4 days of treatment (mean 3.47; SD 2.14). There was a significant difference between treatment and control groups in the incidence of mastitis at calving. Heifers treated with penethamate hydriodide had almost half the risk of mastitis as control heifers. The incidence in treated animals was 22% (12 out of 54) compared to 46% (16 out of 35) in controls (RR = relative risk = 0.49, p=0.0197). No significant difference between groups was observed for the mastitis incidence at 7 days post calving. There was a significant difference between the number of culled animals for mastitis reasons in favour of the treatment group with only one cow being culled compared to 4 animals in the control group (RR 0.17; CI 0.03, 1.12; p=0.07).

Conclusion: This data suggests that, presented with a high yielding group of heifers with a previous history of peri-calving mastitis, preventive therapy with penethamate hydriodide would be likely to be of significant benefit pre-calving.

Funding: Boehringer Ingelheim Animal Health GmbH

459 (2817)

IN VITRO GROWTH INHIBITION OF MAJOR MASTITIS PATHOGENS BY STAPHYLOCOCCUS CHROMOGENES ORIGINATING FROM TEAT APICES OF DAIRY HEIFERS

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Previous studies have shown that naturally occurring intramammary infections (IMI) with Staphylococcus chromogenes have a considerable protective effect against intramammary challenge with Staphylococcus aureus (1). In addition, recent field observations showed that teat apex colonization by S. chromogenes prepartum protects udder quarters against elevated somatic cell counts early post partum in dairy heifers (2). In the present study, the in vitro inhibitory capability of S. chromogenes originating from teat apices of young, end-term, and recently calved dairy heifers towards the most important major pathogens cultured from subclinical and clinical mastitis cases, was tested to explain this finding using a modified cross-streaking method.

Two out of ten S. chromogenes isolates, both originating from different teats from the same heifer, inhibited growth of all S. aureus (n = 5), all Streptococcus dysgalactiae (n = 5), and all Streptococcus uberis (n = 2) under study to a certain extent, however, with decreasing intensity. No meaningful inhibitory activity towards the growth of Escherichia coli (n = 5) could be detected. The minor inhibitions observed within the study were most probably caused by limited availability of nutrients. The more wide-ranging inhibitions, however, suggest the production of antagonistic substances by some S. chromogenes strains, and therefore support the observations of a previous study that indicated a protective effect of teat apex colonization by S. chromogenes in vivo. Other mechanisms explaining the protective effect, however, appear to be present as only a minority of the studied isolates showed detectable inhibition in the test method used.

The results of the present study are in accordance with the findings of others who found that 25% of the isolates of normal teat skin flora of non-lactating heifers were able to inhibit the growth of selected mastitis pathogens (3), and are an indirect confirmation of earlier and more detailed studies describing specific antagonistic substances produced by CNS (4). The findings of this study are promising and could lead to new preventive measures in the struggle against mastitis.


460 (2586)

DEPLETION OF RESIDUES IN EDIBLE TISSUES AND MILK FOLLOWING TREATMENT WITH A NEW CEFQUINOME DRY COW FORMULATION
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Purpose: Cefquinome, a fourth generation cephalosporin, is included in an intramammary preparation for the cure of bacterial mastitis in lactating cows (Cobactan LC). Subsequently, a dry cow formulation (Cobactan DC, containing 150 mg cefquinome per injector) has been formulated for treatment of subclinical mastitis at drying-off and prevention of new bacterial infections during the dry period. The objective of the four studies (I-IV) reported here was to generate data for recommendation of withdrawal periods for edible tissues and milk after administration of Cobactan DC at the therapeutic dose to all quarters of dairy cows at drying off or during lactation.

Methods: (I.) Tissues (fat, muscle, liver, kidney) from 4 cows each were collected at slaughter 2, 7 and 14 days after treatment at drying off. (II.) Plasma was gathered at regular intervals during the first 3 weeks of the dry period from another 6 treated animals. (III.) In a third study with 29 animals, aliquots of each cow's total milk production were sampled at each milking post calving until day 5. (IV.) In the last study designed to determine the residues in milk post partum in cows with a short dry period (<35 days) or after erroneous treatment during lactation, 23 lactating cows were treated. All matrices were analysed for cefquinome with validated routine HPLC methods.

Results: (I.) Maximum residue levels (MRLs) for cefquinome were never exceeded in any of the edible tissues from day 2 to 14 after treatment. Most samples were below the limit of quantification (LOQ) or already below the limit of detection (LOD). (II.) No cefquinome was quantifiable in the plasma samples collected from 4 hours to 21 days after treatment. (III.) All cefquinome concentrations in the milk after calving were below the MRL, mostly even below the LOQ at first milking, and at day 5 post partum all were below the LOD. (IV.) Milk residues were below the MRL from the 51st milking post-treatment in all lactating cows except one (which had levels below MRL from the 65th milking).

Conclusions: Cefquinome was not absorbed systemically after treatment at drying-off. As concentrations were always below the MRL in milk post partum and all edible tissues, Cobactan DC is a highly safe product for the consumer. For cows with a dry period of less than 35 days after treatment or in case of erroneous treatment during lactation, a milk withholding period of 5 weeks post-treatment for milk is recommended.

461 (3223)
“SYSTEMIC” AND INTRACISTERNAL ADMINISTRATION OF CEFQUINOME - TISSUE DISTRIBUTION IN THE ISOLATED PERFUSED BOVINE UDDER
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Purpose: Cefquinome, a broad spectrum cephalosporin, is approved for administration to pigs and cattle. Acute bovine E. coli mastitis is one of the indications for its systemic and intracisternal use. Sufficient antibiotic concentration in the udder is a prerequisite for efficacy. The concentrations in milk and blood in vivo do not necessarily provide an accurate estimation of the local availability. Therefore, the distribution of cefquinome in glandular tissue was studied in the isolated perfused bovine udder.

Methods: Immediately after slaughter, healthy lactating bovine udders were perfused via their blood vessels in vitro with warmed and gassed Tyrode solution. Cefquinome was given “systemically” via the perfusion fluid in concentrations similar to those analysed in blood after intramuscular administration of 1 mg/kg body weight as Cobactan® 2.5% and/or given intracisternally to all udder quarters as Cobactan® LC (75 mg cefquinome). From 4 quarters each per route of administration, perfusion fluid was collected in intervals over 6 hours, udder lymph nodes after 6 hours and glandular tissue in different distances vertical to the teat right up to the udder base after 2, 4 and 6 hours. Cefquinome was analysed in these matrices by high performance liquid chromatography and bioassay.

Results: The administration of cefquinome via the perfusion fluid resulted in median concentrations of 0.1 to 0.4 µg/g in all areas of the mammary gland. After intracisternal treatment, the cefquinome concentration in the udders decreased exponentially with increasing distance from the teat. Already after 2 hours, the parallel treatment on both routes was followed by concentrations above the MIC90 values (0.2 µg/g) of the most common mastitis pathogens in all tissue locations. As with other actives, a high variation in tissue concentrations especially after local administration was noted. This is representative for the individuality even of healthy bovine udders resulting from different milk yield and glandular size. The variability is considered as the “normal” situation during treatment of mastitis. 

Conclusions: The results represent a comparison of the cefquinome levels in bovine udders after systemic and intracisternal treatment. A combination of these routes will obtain maximum antibiotic concentrations in all locations of the gland. This therapy was also clinically and bacteriologically most effective in E. coli mastitis.

462 (1379)
INVESTIGATION ON THE IN VIVO EFFICACY OF RNA INHIBITING PEPTIDE (RIP) IN INTRAMAMMARY TREATMENT OF LACTATING COWS WITH SUBCLINICAL STAPHYLOCOCCUS AUREUS MASTITIS
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RNA inhibiting peptide (RIP) is a heptapeptide that is considered to be a global inhibitor of Staphylococcus (S.) aureus by disrupting quorum-sensing mechanisms. The ability to reduce toxin synthesis and cell adhesion in vitro and to be an effective inhibitor of all strains of S. aureus infection so far tested in vivo indicated the potential of RIP as a therapeutic agent to staphylococcal infections.

A randomised, blinded, placebo-controlled pilot trial investigated the efficacy of intramammary RIP treatment at the recommended dose (1) of 150 µg per quarter in chronically S. aureus infected udder quarters of lactating cows.

Overall, 14 cows (n=7 per group) with chronic S. aureus infection were treated over 3 consecutive days in 12 hour intervals after milking with either RIP (intramammary infusion of 150 µg solved in 10 ml sterile water per quarter; group A; n=13 quarters) or RIP placebo (10 ml sterile water; group B; n=12 quarters). For bacteriology, the number of colonies (CFU) was evaluated weekly in quarter milk samples before and after therapy and at 12 hour intervals over 3 consecutive days during therapy. In addition, the somatic cell count (Fossomatic), N-acetyl-glucosaminidase (NAGase) and the electronic conductivity (Mastitron) were determined.

There were no significant differences between the two treatment groups. A significant reduction (p < 0.05) for the mean of CFU was achieved during the therapy, however this effect was observed in both groups. The mean number of CFU (log 10) in group 1 was in milk samples taken before therapy 2.01, during therapy 1.49 and after therapy 2.00; corresponding mean values in group 2 were 1.86, 1.30 and 1.74. Mean somatic cell count (log 10/ml milk) ranged in group 1 around 5.60 and in group 2 around 5.90 before and after therapy. The NAGase and conductivity mean values were not significantly changed in both treatment groups.

Contrary to data published (1), no efficacy of RIP on subclinical S. aureus infection was found in this pilot study. Interestingly, the infusion into the udder lead to a significant reduction of colony forming units of S. aureus during therapy in both groups, however there was no cure of S. aureus achieved for investigation of milk samples taken subsequently.

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463 (2581)

SUSCEPTIBILITY SURVEY OF MAJOR BOVINE MASTITIS PATHOGENS TO CEPHALEXIN IN FRANCE AND IN GERMANY

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Mastitis control programs encompass widespread use of antibacterial drugs. During the post-marketing lifespan of a commercially available intramammary drug, a periodic monitoring of pathogens sensitivity pattern is desirable. The minimum inhibitory concentrations (MICs) of cephalexin were determined against 120 first-intention isolates of bovine mastitis using an agar dilution method based on National Committee for Clinical Laboratory Standards (NCCLS) methods. Forty isolates, each of Escherichia coli (Ec), Streptococcus uberis (Su) and Staphylococcus aureus (Sa), were recently isolated from France (1999 - 2000) and Germany (2003).

The 60 French strains were sourced from the Veterinary Pathogens culture collection (Vetpath, CEESA) and all 120 were high quality isolates, that is taken prior to any antibiotic treatment and for which history (source identification, date) was available. The MIC90 - MIC50 values (µg/ml) were 8 - 4 against Ec, 4 - 2 against Sa and 0,25 - 0,25 against Su respectively. These values very closely compare, even were lower, with the previous results of MIC determination performed at the request of Virbac in 1994, where MIC90 - MIC50 values were 8 - 4 against Ec, 8 - 4 against Sa and 0,5 - 0,25 against Su respectively (n=75 for each pathogen, all french strains) (ref 1) or meanwhile reported by others (ref 2&3). The pharmacokinetics of cephalexin in milk and parenchyma have been investigated. Twelve hours after completion of the full treatment (4 infusions in total at 12 hours interval, n=6 cows), the mean cephalexin concentration was 11,7 mcg/ml (ref 1). After a single intramammary infusion of radiolabelled cephalexin (4 quarters treated, n=3 cows), parenchyma biopsy samples taken at 12 and 72 hours post dose showed a progressive tissue concentration with cephalexin levels of 5,6 and 8,3 mcg equivalent/g respectively (ref 1). Cefalexin, a first generation cephalosporin, has a bactericidal time-dependent activity. Rapidly achieved and lasting efficient concentrations of cephalexin in milk and parenchyma remain in line with the recently reported MICs against the major bovine udder pathogens.

We conclude from these results that after 10 years of marketing in France, the sensitivity pattern of cephalexin against the three major bovine mastitis pathogens is not modified.

References: 1 Registration dossier - unpublished data; 2 Morvan H. and Parez, Le Point Vétérinaire, 1997; 3 Guérin-Faublée and coll, The Veterinary Record, 2003
Funding: Study sponsored by Virbac

464 (2623)

AUTOMATIC MILKING CONDITIONS AND EFFECTS OF INCOMPLETE MILKINGS ON MILK YIELD

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Goal of the study: Depending on the frequency of occurrence and the amount of milk left in the udder, incomplete milkings (IM) are assumed to cause a marked reduction in yield. This study was performed to evaluate the influence of IM on milk yield under conditions of automatic milking.

Material and methods: A group of 40 high-yielding German Holstein Frisian cows at different lactation stages and numbers was milked with a robotic milking system (DeLaval, voluntary milking system; VMS; 42 kPa vacuum, 60 cycles/min pulsation rate, 65% pulsation ratio). VMS identified a milking as incomplete when the actual yield did not reach a certain level of the expected amount (60% at quarter and/or 80% at cow level). Over 400 days, survey was carried out every 20 days (test day; TD), and each session included 24 hours of sampling where clusters were attached manually. Overall, 35762 milkings were analysed (including 4560 incomplete ones).

Results: In 70% of all cases, only one quarter of the udder was milked incompletely. Strikingly, 50% of these were rear ones. This suggests that the majority of IM was caused by limited accessibility of the rear quarters (technical difficulties). On the other hand and considering cow-individual factors, 5 cows out of 40 (=12.5%) on average were responsible for 44% of all IM. During TD, the cluster attachment was done manually, so that no IM could occur. The influence of IM on average yield was determined by comparing calculated weekly yields (WY = TD values x 7 days) with the actually recorded ones. For TD 1 - 10, the calculated WY was 5891 ± 570 kg and the recorded WY 5998 ± 635 kg with 9.7% IM. Corresponding values for TD 11 - 20 were 6357 ± 998 kg, 5763 ± 906 kg and 12.1%, respectively. Thus, the results did not indicate any significant impairment on milk yield by IM.

Implications: The results suggest that IM occurrence is conditioned by technical (reduced accessibility to rear quarters) and cow-individual circumstances. Here, the effects caused by a mean of approx. 10% IM were compensated by the increased milking frequency (2.86 times/cow/day) and shorter inter-milking intervals so that no significant differences in milk yield could be detected.

465 (2625)
SECRETORY ACTIVITY OF HEALTHY AND DISEASED UDDER QUARTERS UNDER THE CONDITIONS OF AUTOMATIC MILKING
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Goal of the study: Milk secretion is, in addition to factors such as genetics, feeding and milking regime, mainly determined by the udder health status. This is widely accepted for conventional milking. The present study compares the secretory activity of healthy and diseased quarters under conditions of automatic milking in order to estimate the importance of udder health in this context.

Material and methods: A group of 40 high-yielding German Holstein Frisian cows at different lactation stages and numbers was milked with a robotic milking system (DeLaval, voluntary milking system; VMS; 42 kPa vacuum, 60 cycles/min pulsation rate, 65% pulsation ratio). Over 400 days, survey was carried out every 20 days (test day; TD), and each session included 24 hours of continuous sampling. The mean secretory activity of all quarters (n = 6194 samples) was determined in relation to the udder health category (IDF). A detailed analysis of a subgroup (2121 samples from 44 healthy quarters with a somatic cell count <100000 cells/ml and 113 diseased quarters with a cell count level >200000 /ml) was performed. Significances were calculated using Ryan-Einot-Gabriel-Welsch multiple range test and Student t-test.

Results: The overall secretion rate of normally secreting quarters (317 g/h; n = 3212 samples) differed significantly (p<0.05) by 22% from that of mastitic ones (248 g/h; n = 687). For the subgroup, the milk secretion rates [g/h] for the intervals <6h, 6 - 8h, 8 - 10h, 10 - 12h and >12h were 385 ± 148, 318 ± 134, 276 ± 116, 274 ± 120 and 238 ± 102 for healthy and 352 ± 188, 265 ± 122, 252 ± 133, 213 ± 116 and 200 ± 103 for diseased quarters, respectively. All comparisons among milking intervals resulted in significant differences (p<0.05) in secretory activity between healthy and diseased quarters, with the exception of the interval <6h. The analysis of variance confirmed the significance of the different milking intervals for both healthy and diseased quarters. Both quarter groups showed secretory reductions of approx. 40% between interval <6h and >12h.

Implications: Unlike physiological influences, the mean yield reduction at quarter level of approx. 20% due to mastitis was not compensated by increased milking frequencies (2.86 milkings/cow/day).

466 (2621)
FLOW CYTOMETRIC DIFFERENTIATION OF SOMATIC CELLS IN MILK
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Goal of the study: Differential cell count in quarter milk samples is an important means to determine udder health status. Flow cytometry as a method to detect different cell populations might replace time-consuming microscopy.

Material and methods: This study compared percentages of lymphocytes, polymorphonuclear neutrophils (PMN) and macrophages obtained by microscopy and flow cytometry of quarters with a somatic cell count (SCC) of up to 200000 cells/ml. Overall, 94 samples of German Holstein cows from two farms were prepared...
for microscopy (10 minute centrifugation at 1400 x g and sediment spreading on slides) before 200 cells per sample were evaluated. For flow cytometry, cells of the same samples were incubated with a DNA-marker. Before starting the investigation, the regions of the different cell types in dotplots were defined by using specific antibodies. SCC was determined twice by Fossomatic® and according to this, three groups (G1-G3) were established (G1: <50000/ml, n=37; G2: 50000-100000/ml, n=29; G3: 100000-200000/ml, n=28). All in all, 5000 lymphocytes, PMN and macrophages were counted and their percentages calculated. Results of both methods were compared within each group.

Results: Regarding results for PMN, a comparability of both methods was given. PMN percentages obtained by the Fluorescence Activated Cell Sorter (FACS®) and by microscope in group 1 (FACS: 30.69%; microscope: 28.84%) differed significantly (p=0.001) from those in group 2 (FACS: 46.31%; microscope: 49.28%).

Implications: PMN are the first line of defense against udder infections and might therefore turn out to be the most important parameter. It is possible to determine udder health status by flow cytometry, especially in lower SCC-groups. With further investigation, a substitution of microscopy by flow cytometry seems feasible.

467 (2611)
INTERACTION BETWEEN INFLAMED AND HEALTHY BOVINE UDDER QUARTERS
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Goal of the study: At present, evaluation of cyto-bacteriological data and other milk constituents to define udder health status is based on the assumption that any reaction of these parameters is strictly single quarter-related (quarter independence). The present study was designed to investigate the reactivity of all quarters within a mammary gland when only one single quarter develops a mastitis.

Material and methods: 27 German Holstein cows at different lactation stages and numbers with 4 healthy quarters (<100000 cells/ml) were selected. Milk constituents (somatic cell counts, NAGase, electrical conductivity, galactose) in quarter foremilk (OFM) and quarter composite (QCM) samples were analysed thrice before (-3, -2, -1) and at the event (0) a mastitis in one single quarter occurred (>100000 cells/ml). The analysis of variance for not normally distributed, paired data by Friedman was applied to determine compositional changes in the three healthy quarters (group 1) and the diseased one (group 2). In order to reach a comparability of all data sets, parameter changes were expressed in percentage to the lactation stage corresponding physiological reference.

Results: Mean percentage values for all parameters (100% = corresponding physiological reference) in relation to milk fraction, observation stage and udder health group revealed that quarters diseased at sampling date 0 (group 2) showed higher levels over all observations. For healthy quarters (group 1), significant increases also occurred (p<0.05), especially in the transition from observation -1 to 0. Mean ratio values >1.0 as seen for this period, support these results.

Implications: The assumption that the 4 quarters of the bovine udder function as independent units seems to be based on the anatomical structure. However, other studies show interactions between udder quarters concerning milk yield (depression vs. compensation). Moreover, these effects are accompanied by compensatory growth of glandular tissue. At least concerning cell count levels, significant changes were observed for two milked quarters per cow after the other two were left unmilked for two days. Therefore, these findings support the evidence that udder quarters interact with each other. This implies that modern mastitis diagnostic procedures should be judged in relation to these results.

468 (1235)
SETTING UP OF A COMPUTERIZED TOOL OF ECOPATHOLOGICAL APPROACH OF MASTITIS IN DAIRY HERDS
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Mastitis is a typically subclinical disease of production, due to various factors directly or indirectly in relation with bacteria, animals or their environment. His economical effects are still rather extremely serious. Different reasons have led us to set up an ecopathological approach namely ASM (Audit de santé mammaire) to take into account all the factors involved in the mastitis problem in dairy herds. The first is to have a better understanding of the respective role of the risks factors of mastitis. The necessity of a more preventive than curative approach was a second justification. The third one was economical. The milk sector represents in Belgium a very important part of the agroalimentation sector. Finally, despite real progress in continuing education, there is still an increasing demand to follow up the dairy producers. The ASM setting up has been the object of a wide review of literature. A critical analysis of the available information followed by two phases of experimentation in the field has allowed us to refine progressively the data of the survey in order to optimize the collection of information. The work reveals some characteristics: real conviviality, simplified understanding (boxes to tick) in order to facilitate future numerical treatment, an understanding as objective as possible thanks to specific indices (indices of comfort, level of cleanliness...). The questionnaire has been built up as a structured and hierarchical approach of risk factors, primary or linked to six items: general
information on the herd, clinical and subclinical data of the mammary health, milking, housing, the
zoootechnical and therapeutical management of the mammary infections, the producer's opinion. In order to
ensure better quality of the collected data but also to facilitate their treatment and the analysis of the
underlying results, we have developed a micro-computerized support for the coding of the questionnaire. This
approach allows the optimization of the process of data collecting, to standardize the coding and to facilitate
the use of ASM. The interface corresponds to a computerized copy of the questionnaire. This configuration
allows future automatization of any possible statistical requirements.

469 (2239)
THE EFFECT OF GENOTYPE ON CLINICAL CHARACTERISTICS AND PERSISTENCE OF BOVINE S.
AUREUS MASTITIS
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The purpose of the present study was to find out if the type of mastitis causing S. aureus has any effect on the
clinical characteristics of mastitis or persistence of the infection. A total of 116 dairy cows (134 quarters) with
intramammary infection caused by S. aureus were studied. The cows were from 70 herds located in Southern
Finland in the practice area of the Ambulatory clinic of the University of Helsinki. The attending veterinarian
recorded the history and clinical data from the cows and took aseptic milk samples before treatment. Quarter-to-quarter transmission and persistence of S. aureus infection were followed by examining growth of S.
aureus from the samples taken during follow-up visits two and four weeks later. Mastitis was classified as
subclinical, mild clinical or serious clinical based on the physical examination and somatic cell count. Cows
infected with betalactamase negative isolate were treated with penicillin G (n = 69) and those infected with a
positive isolate with other betalactam antibiotics (n = 47). Gram-positive, catalase positive and rabbit plasma
coagulase positive cocci were confirmed as S. aureus by PCR amplification of thermonuclease (nuc) gene.
The bacterial isolates (n = 217) were typed using pulsed-field gel electrophoresis (PFGE). Visual comparison
of the 217 PFGE typed S. aureus provided 22 pulsotypes (A-V). Pulsotypes F to V were sporadic, and they
were isolated from individual cows only. The common pulsotypes A to E consisted of 2 to 5 closely related
clones and were shared by multiple herds, and these pulsotypes infected 90% of the cows. In 77% of the
herds only one pulsotype was found. The majority of the cases (46%) were mild clinical, 25% were severe
clinical, and 29% subclinical. One common pulsotype was associated with severe signs and strong udder
response, but short persistence. Spreading of some S. aureus pulsotypes from the originally infected quarter
to a previously healthy quarter in seven cows was noticed. Same clone was isolated if the cow had several
infected quarters. Results support the existence of differences in virulence potential among S. aureus strains
causing bovine intramammary infection.
Funding: MAFF Finland, WE Foundation

470 (3359)
RESULTS OF BACTERIOLOGICAL ANALYSES OF CLINICAL BOVINE MASTITIS AFTER SIX YEARS OF
THOROUGH FOLLOW-UP ON A SINGLE FARM
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A systematic analysis of milks from quarters affected by clinical mastitis was carried out over a six year period
on the same herd. A high incidence of clinical mastitis was observed in this farm of eighty dairy cows, situated
in Haute-Savoie, France (near Geneva); the somatic cell counts, however, remained low throughout all the
period: 110 000/mL in the bulk milk (used for the production of cheese from non pasteurised milk).
For each clinical mastitis (defined as a change in the appearance of the milk e.g. clots detected visually, with
or without a change in quarter appearance or general symptoms), a sample was taken aseptically,
refrigerated and brought to the same laboratory within 48 hours. Then, a bacteriological analysis was carried
out without enrichment. Among the 244 samples analysed, 27 different germs were isolated at least once.
The relative proportion of the germs changed over time according to management practices (see below).
Overall, between the period 1997-1998 and 2001-2002, the incidence of clinical mastitis decreased from 60%
to 45% of cows affected by at least one mastitis during the milking period; in closer detail, Staphylococcus
aureus practically disappeared (from 16% to 3% of the mastitis cases [p = 0.002]), the other Staphylococcus
species (coagulase negative) decreased but Streptococcus uberis and the enterobacteriae doubled (from
23% to 52%)
In the 1999-2000 period, the main management practices modified on the farm were: 1) Improved hygiene
with the disinfection of the teat ends just before milking; 2) Improved ventilation of the building; 3) Extended
grazing period in spring and summer. Furthermore, between 1997 and 2002, on this farm: 1) Most cases of acute mastitis (6/8 were associated to
coliform bacteria; 2) However, the opposite was not true, since the great majority of cases of coliform mastitis
(26/32) were not acute; 3) The breeders collected the samples by themselves without contaminating them
(0.5%); 4) No germ was isolated in about 25% of the samples.
The monthly analyses on bulk milk carried out in the last three years have shown that the farm milk was almost free of Staphylococcus aureus (< 10/ml).

**Funding:** Intervet

**471 (1724)**

**RELATIONSHIP BETWEEN YEAST MASTITIS IN DAIRY COWS AND ISOLATION OF YEASTS FROM THE MILKING MACHINE**

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Yeast mastitis as a herd problem is quite often thought to be connected with an insufficient sanitation of the milking machine, especially when yeasts were isolated from swab samples taken from the milking machine. However, because during routine microbiologic examination, yeasts are usually not differentiated down to the species level, it is not known whether or not yeast species found in mastitis cases resemble those found in the milking machine. Therefore, the aim of this study was to differentiate yeasts from mastitis cases (clinical and subclinical) and from swab samples taken at various locations of the milking machine (cluster, milk line). One hundred and forty-four yeast isolates (75 isolates from milk samples and 69 isolates from swab samples) from 22 dairy herds were used. Species identification was carried out using the ID 32 C® identification system (bioMérieux company, Nürtingen, Germany). Furthermore, the growth behaviour on rice agar extract and on "Hamburger-Test"-Agar (HT-Ägar) at 37°C was tested. In case no species identification was obtained with this assay system, conventional assimilation and fermentation assays were applied. From 144 yeast isolates, species identification was possible in 132 cases. Twelve different yeast species were identified in milk samples and 15 yeast species in swab samples, respectively. The species found most frequently in milk samples were Cryptococcus curvatus, Candida (C.) kruzei, C. famata, C. tropicalis and C. rugosa. The predominating species in swab samples were C. parapsilosis, C. norvegensis and Crypt. curvatus. From 46 out of 75 milk samples (61.4%), yeast isolates had the ability to form a pseudomycelium and grow at 37°C. However, only 4 of those isolates (8.7%) were able to assimilate lactose. Thirty-two out of 69 isolates from swab samples (46.4%) had the ability of pseudomycelium formation and growth at 37°C of which 14 (43.8%) had the capability to assimilate lactose. In 6 of the 22 dairy farms (27.3%), yeast species from swab samples were also found in milk samples. Our results suggest that due to their culture behaviour, many of the isolated yeast species have the potential to act as udder pathogens. Furthermore, milking machines contaminated with yeasts might act as a vector of transmission for those udder pathogens.

**472 (1337)**

**THE EFFECT OF ‘BLITZ’ TREATMENT OF STREPTOCOCCUS AGALACTIAE WITH CEFTIOFUR SODIUM ON BULK TANK SOMATIC CELL COUNT AND TEST-DAY MILK YIELD IN A DAIRY HERD IN IRAN**

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Streptococcus agalactiae (S. ag.) is a major cause of subclinical mastitis in dairy cows. High mammary tissue and milk concentrations of ceftiofur following the intramammary administration have been previously demonstrated. The objective of this study was to determine the effect of ceftiofur sodium as a therapeutic for blitz treatment of S. ag. on bulk tank somatic cell count (BTSCC) and milk yield of infected cattle in a large dairy herd. Based on the bulk tank somatic cell counts and bacteriological cultures, S. ag. was identified as the major cause of mastitis in a 300-cow dairy herd in southeast of Tehran, where dry cow therapy and post milking teat dipping were improperly used. Composite milk samples were collected aseptically from all the 252 lactating cows, S. ag. being isolated from 77 (30.55%) samples. The infected cows received three intramammary infusions of 125 mg of ceftiofur sodium (Excenel®, Pharmacia) in each quarter at 24-h intervals. Afterwards, milk samples from 24 (31.17%) treated cows were randomized to be cultured on two occasions following the treatment and S. ag. was isolated from just three cows. Bulk tank milk samples were collected bi-monthly for a period of 3½ months before the treatment and for a similar period after that to determine BTSCC and culture. BTSCC was measured using direct microscopic counting, and microbiological procedures were conducted in accordance with National Mastitis Council standards. Two-sample t-test showed that the arithmetic mean of BTSCC after the treatment (251,365 cells/ml) was significantly (P<0.001) lower compared with that before treatment (610,521 cells/ml). Paired t-test showed that 5 days after the treatment, the average of test-day production of treated cows was significantly higher (26.45 kg) than that of a test-day production before the treatment (25.19 kg) (P<0.01). In addition, by Fisher's exact test, we found that there was a significant association between the blitz treatment and reduced detection of S. ag. of the bulk tank samples (from 85.71% of bi-monthly samples prior to the treatment to 14.28% after that; P=0.014). A positive correlation between BTSCC and bulk tank milk concentration of S. ag. also proved to be significant (r=0.537, P<0.05). We concluded that intramammary administration of ceftiofur at the mentioned dose is efficacious to decrease the prevalence of S. ag. in dairy herds and could be used to reduce BTSCC and raise milk production in infected cows.

**Funding:** University of Tehran

**473 (3174)**

ARE HOMEOPATHICS ABLE TO REPLACE ANTIBIOTICS IN THE THERAPY OF BOVINE MASTITIS? A
PLACEBO CONTROLLED RANDOMIZED DOUBLE-BLIND TRIAL
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The critical discussion about general application of antibiotics in the control of bovine mastitis is increasing. Complementary therapy measures are demanded, but few studies are proving their efficacy, yet. In a previous study conducted in the same organic dairy herd with about 200 cows, clinical cure rates of mastitis of 51% after homeopathic treatment compared to 60% after antibiotic treatment were calculated (GARBE, 2003).

To evaluate the net efficacy of the homeopathic remedies, a placebo controlled randomized double blind study was conducted. 68 cows (77 affected quarters) in the homeopathic treatment group (HOM) and 60 cows (67 affected quarters) in the control group (CON) matched the inclusion criteria. In case of clinical mastitis, the HOM group was treated by an orally applied combination of 3 to 4 remedies (depending on clinical symptoms according to homeopathic prescription guidelines). Cows were treated two times a day with 5 ml of a mixed dilution until clinical recovery. Besides the obligatory quarter milk sampling, no other measures were made. In cases of no recovery within 7 days (therapy failures), the animals received open consecutive treatment with the same homeopathics.

Overall, a total of 63% (N=43) of treated cows and 61% of quarters (N=47) in group HOM and 67% (N=40) of cows and 63% (N=42) of quarters in group CON, respectively, could be released to production without consecutive additional treatment (p>0.05). Considering recurrences, the clinical cure rate after 21-28 days was 43% (N=29) and 42% (N=32) for HOM and 50% (N=30) and 46% (N=31) for CON (p>0.05) respectively. Furthermore, the cure rate in both groups was about 20% higher in cases of unspecific mastitis compared to cases with infections with major pathogens (p<0.05). The cure rate increased during the second half of the investigation by about 20% (p<0.05).

The results suggest that the effects of therapy are caused by self healing and not by the homeopathic remedies. The increasing cure rates during the time of investigation are obviously effected by improved environmental and management factors. This underlines the importance of preventive measures in the control of bovine mastitis. Moreover, the clinical and bacteriological results suggest that the efficacy of general use of antibiotics in mastitis control has to be discussed in a more sophisticated way and has to be challenged.

Funding: Federal Agency for Agriculture

474 (964)
RELATIONSHIP BETWEEN TEAT INJURY AND SUBCLINICAL MASTITIS PREVALENCE IN DAIRY COWS IN A HERD OF SÃO PAULO STATE, BRAZIL

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The subclinical mastitis is one of the main diseases in the dairy herds. Works accomplished in Brazil using the somatic cells count (SCC) as tool of mastitis diagnosis, show a disease prevalence of 40%, (animals with SCC over than 280 thousand cells/ml of milk are considered infected). With the objective of identifying causes of mastitis, the integrity of the teats of the animals was checked along with quantification of individual SCC. We used 593 dairy cows in lactation coming from a commercial dairy farm located in the state of São Paulo. The teats were classified in score 0 and 1 (0: teats without lesion and 1: teats with lesion). The average SCC of the herd was 234 thousand cells/ml, the average linear SCC score (LS) was 2,25 and the prevalence was 12,5%. Animals with lesion in the teats had averages of 2,88 and 333, for LS and SCC (x 1.000), respectively. The animals without lesion had inferior averages (p<0.05) of 2,04 and 203 for LS and SCC (x 1.000), respectively. The prevalence in animals with score 0 and 1 was of 3% and 24%, respectively. The observed results show the importance of the integrity of the teats in the prevention of new infections. The maintenance and adjustment of the milk equipment are fundamental for the maintenance of the teat integrity.

Key Words: Somatic Cells Count, Mastitis, Teat score.
Funding: FAPESP e Clínica do Leite/ESALQ

475 (2431)
MILK IODINE RESIDUES: AMS VERSUS CONVENTIONAL

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Extensive research has shown the benefit of using iodine-containing teat dip products in the reduction of new intramammary infections in dairy cows. Their use has been shown to increase the iodide concentration in milk by up to 150 ppb in conventional milking systems (Hemling “Iodine Residues in Milk”, XXI World Bulatrics Congress, Punta del Este Uruguay, December 4-8, 2000). The use of an automated milking system (AMS) could possibly provide new risk factors that affect the level of iodine residues. Under an automated milking system, cows will be milked more frequently than under a conventional milking system and pre-milking teat preparation is automated. We report here data on iodine residues from milk collected during a trial evaluating udder health and teat condition during the transition from conventional to AMS milking while using a high emollient, low iodine teat dip (Proactive Plus™, DeLaval). Forty lactating cows and heifers from a high yielding dairy herd were randomly allocated to control and AMS
groups (VMSTM, DeLaval), each group containing both heifers and multiparous cows. In the AMS group, robotic milking was initiated during the study period, while in the control group conventional milking was continued throughout the duration of the study period. Composite milk samples were collected from both groups before and after converting to AMS milking. Iodine levels were determined by HPLC method (Food and Dairy Chemistry Laboratory, University of Guelph, Guelph, Ontario, Canada).

Pre-trial Iodine in Milk (ppb): control 76.8 vs AMS 72.4
Trial Iodine in Milk (ppb): control 74.6 vs AMS 75.1

Results showed low iodine levels in both the Conventional and AMS groups for both the trial and pre-trial period. (Change in iodine concentration, pre-trial vs. trial, was not statistically different between groups, p=0.50). There were no differences observed between treatments. The iodine levels are well below the recommended maximum of 500 ppb level. The low iodine levels are likely the result of the low iodine content (0.15%) of the Proactive Plus teat dip and good teat washing by the VMS during the pre-milking procedure.

Funding: DeLaval

476 (2075)
FREE IODINE AND THE GERMICIDAL ACTIVITY OF IODINE TEAT DIPS
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Iodine teat dips have been widely used since the 1960's as post milking pre-milking germicides. Commercial compositions range in iodine content from 0.05 to 1.0% available iodine. Murdough et al has shown that germicidal activity is not correlated with the available iodine level. It has been shown that increases in free iodine level will increase the germicidal activity of pre- and post milking teat dips. New technology allows ready to use compositions to be prepared with even higher free iodine levels. We report here a natural exposure trial comparing the efficacy of two 0.25% available iodine teat dips differing in the free iodine level.

A natural exposure trial utilizing a positive control was performed following NMC guidelines in Argentina using two groups of 48 cows each. The groups were assigned according to parity, days in milk, and teat condition. A whole herd micro evaluation of each quarter was performed at the beginning of the trial and every two weeks during the trial. The trial ran from December to September through some very hot weather and through the winter months. The teats of the cow were cleaned and dried before milking and dipped with the appropriate teat dip after milking. Teat condition was also evaluated every two weeks during the trial.

The positive control Teat dip #1 (Della Care, DeLaval) had an available iodine content of 0.25% with 2% glycerin and a free iodine value of 5-8 ppm at 25°C. Teat dip #2 (Della Care with I-tech2, DeLaval) had an available iodine content of 0.25% with 2% glycerin and a free iodine value of 12 - 16 ppm at 25°C. A total of 100 infections (33 major pathogens, and 67 minor pathogens) were observed for the cows dipped with Teat Dip #1. A total of 45 infections (14 major pathogens and 31 minor pathogens) were observed for the group of cows dipped with Teat Dip #2. A significant reduction in infection rate was found for the Teat Dip #2 versus the positive control for: total infections (p = 0.001), major pathogens (p=0.01), minor pathogen (p= 0.001), Staph. aureus (0.01 ), and Strep. ag (p=0.2). Teat dip #2 gave slightly better teat end condition (p=0.21), but the teat condition was not statistically different from teat dip #1. These results confirm the benefit of higher free iodine in iodine teat dips.


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477 (1381)
MITIGATION OF TEAT END CONDITIONS UNDER ADVERSE WEATHER CONDITIONS
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Healthy teat skin has been shown to reduce colonization by mastitis causing bacteria and to adversely affect milking time and milk yield. Adverse weather, such low temperatures, low humidity and wind chill are known to adversely affect teat condition. Under low temperature conditions some producers either stop dipping, or dip and blot dry in order to reduce the potential for frozen teats or wind chill induced chapping. Alterations in teat dip properties may allow these products to be used under adverse weather conditions. Recently several products with elevated propylene glycol levels (70-75%) have been introduced for this application, but no clinical data has been presented to support their teat conditioning benefit.

Three clinical teat conditioning trials were performed to determine which environmental and teat dip compositional parameters have the greatest influence on teat condition. Teat dip compositions varied in germicidal type, amount of emollient, and drying time. The trials were performed in New York, USA, Iowa, USA and Chile during the winter time and during periods of excessive rain. A total of nine teat dips were evaluated in the combined trials. Seven of the dips contained between 0.15% and 1% iodine. Two dips contained 1% fatty acids. The dips contained between 5 - 75% propylene glycol or 3 to 50% glycerin. Tactile and visual observations were used to measure the teat condition once or twice each week during the trial. An ordinal scale was used to score the teat condition. Separate scores were measured on skin condition, teat
end condition, and, in one trial, teat orifice condition. Temperature, rainfall, and relative humidity data was collected during each trial. The statistical analysis of the ordinal data was performed using the Kruskal-Wallis ANOVA or Mann-Whitney U test.

For teat skin condition, the most important factor found in all three trials was that teat dips containing glycerin gave better skin condition than dips containing only propylene glycol. Example: Triumph (1% iodine, 50% glycerin) gave better skin condition than Derma Kote (0.5% iodine, 74% propylene glycol) with a p value of 0. For teat end condition, fast drying teat dip gave the best teat end condition. Example: Triumph gave better end condition than Derma Kote with a p value of .006. The teat orifice was not affected significantly by the different teat dips.

Funding: DeLaval Inc.

478 (2635)
FIELD RESULTS OF THE SUBCLINICAL INTRAMAMMARY INFECTIONS THERAPY EFFICIENCY
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In the period from 1993 to 2000, 3475 individual quarter milk samples on field were taken for the bacteriologic culturing and drug sensitivity examination. 57% mammary quarters were subclinically infected with the contagious mastitis pathogens (0,4% with Str. Agalactiae and 57% with S. aureus), 30% with the environmental pathogens and 13% with the minor pathogens. 3% of the cow samples were negative. In the beginning there were no changes in the infection rate of most problematic subclinical intramammary infections (IMI) with S. aureus (63%). In 1995 a three-day parenteral and intramammary effective antibiotic treatment of all subclinical selective dry treatment and clinical IMI was introduced. Monthly individual somatic cell count (SCC) was set up as screening test for the subclinical IMI. In the following three years the infection rate with S. aureus decreased by 34%, practically there were no subclinical IMI with Str. Agalactiae and proportional infection rate with environmental pathogens and minor pathogens increased by 25% and 18%. The subclinical IMI has been treated according to the drug sensitivity examination results. In vitro 70% of isolated S. aureus, 84% of environmental pathogens and 92% of minor pathogens were sensitive to the amoxicillin clavulanic acid. 97% of isolated S. aureus, 85% of environmental pathogens and 96% of minor pathogens were sensitive to the cephalaxin. 93% of isolated S. aureus, 72% of environmental pathogens and 92% of minor pathogens were sensitive to the rifampicin. The combination of penicillin, lincomycin and neomycin is in vivo the most efficient combination for treating clinical IMI in our conditions. If there is no response to the therapy in 24 hours, the combination is replaced with kanamycin, rifampicin or tylosin. The therapy success of subclinical and clinical IMI is controlled with initial screening of individual cow SCC. The recovery rate of subclinical IMI with S. aureus is 57,4%, with environmental pathogens 64,5% and minor pathogens 71,9%. The disappointing fact is that 42% of cows recovered from S. aureus got subclinical IMI in the next lactation and 11% in the second lactation. The reinfection rate of cows recovered from environmental pathogens is 43% in the next lactation and 53% in the second lactation and for cows recovered from minor pathogens 52% is in the next lactation. The reinfection rate is lower in farms with relatively good management but it is still too high.

479 (1253)
ETIOLOGY AND PREVALENCE OF SUBCLINICAL MASTITIS IN DAIRY COWS AND ITS ASSOCIATION WITH SEASON, PARITY AND LACTATION STAGE IN THE IZMIR PROVINCE IN TURKEY
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This study was performed between January 2000 - July 2001 using a total of 3532 samples from the quarters of 859 dairy cows between the ages of 1 to 6 years to determine the etiology and prevalence of subclinical mastitis in the Izmir province. In addition to that, the association between seasons, parity, lactation stage and the prevalence of subclinical mastitis was evaluated. The quarter milk samples with a result of California Mastitis Test (CMT) = +1 were further tested for bacteriological examination and those which tested positive were defined as subclinical intramammary infection. 22.1% of the quarter milk samples (761) found to be CMT= +1. 74.4% of the 761 were found positive at the bacteriological examination where the following microorganisms were isolated. Staphylococcus aureus (28.8%), Coagulase-negative staphylococci (21.1%), Streptococcus uberis (11.1%), Gram-negative bacilli (7.7%), Streptococcus agalactia (7.3%), Gram-positive bacilli (6.3%), Streptococcus dysgalactia (5.7%), Corynebacterium bovis (5.7%), Enterococci (4.1%), Fungi (1.2%), Pseudomonas spp. (1%). The prevalence of subclinical mastitis was significantly higher in winter than in the other seasons. The prevalence of subclinical mastitis caused by Gram-negative bacilli was significantly lower in the cows with lactation age of 5 and 6, and significantly higher in the cows in the first two months of lactation.

Results indicate that since the season significantly influences the prevalence of subclinical mastitis, preventive measures should be taken accordingly. Subclinical mastitis caused by gram-negative bacilli should be taken into consideration in young cows and cows which are at the beginning of their lactation period.

Key Words: subclinical mastitis, parity, stage of lactation, seasons.
480 (912)

TREATMENT OF SUB-CLINICAL BOVINE MASTITIS CAUSED BY STAPHYLOCOCCUS AUREUS USING SYNLOX®
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The purpose of the study was to evaluate the efficacy of treatment of subclinical mastitis in dairy cows, caused by the Staphylococcus aureus bacteria strain. Infected animals were treated with Synlox®, which contains amoxycillin and clavulanic acid. In accordance with the manufacturer's instructions, the treatment was applied intramuscularly and intramammary. In total, 61 mammary glands of 37 cows were treated. On average, bacteriological efficacy of the treatment was 51.3%. With animals with one infected mammary gland the efficacy was 69.9%, considering that such animals represented 56.7% of all animals included in the study.
Keywords: veterinary medicine, mastitis, Staphylococcus aureus, treatment, amoxycillin, clavulanic acid

481 (5047)

A SYSTEMIC TREATMENT OF ACUTE COLIFORM MASTITIS WITH ENROFLOXACIN IMPROVES THE GENERAL HEALTH STATUS OF THE DAIRY COW
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Acute mastitis caused by coliform bacteria occurs increasingly in lactating cows and deteriorates severely the general health status along with important losses e.g. caused by endotoxic shock. As the benefit of a systemic antimicrobial therapy to control such a disease condition remains a matter of controversy, we decided to evaluate, under both experimental and field conditions, the response of an intravenous treatment with enrofloxacin.
Cows were inoculated intramammary with a pathogenic E. coli and enrolled into 4 experimental groups as soon as at least one of the following inclusion criteria were met, i.e. increased rectal temperature (RT, >39.5°C), increased local clinical score (LCS, >2), drop of milk yield (MY, >30%), and depressed general behaviour. Twelve cows remained untreated (group 1: controls) whereas others were treated intravenously with enrofloxacin (Baytril™, 5 mg/kg, 2 days; group 2: n= 19) or intramuscularly with ceftiricline (Cobactan™, 1 mg/kg, 2 days; group 3: n=10). Cows of group 4 received three intramammary applications of ceftiricline (75 mg/quarter), 12 hours apart. Bacteriological cure rates were 91.7% and 100% for the controls and the cows treated with enrofloxacin, respectively. For the ceftiricline groups cure rates were 95%. In comparison to the controls, enrofloxacin treated cows recovered better with significant improvements of LCS, RT and MY. Enrofloxacin treated cows scored better than ceftiricline treated cows for all efficacy criteria assessed. The efficacies observed in the above mentioned experimental study for the therapy regimens of group 2 (enrofloxacin, intravenous) and group 4 (ceftiricline, intramuscular) were confirmed in clinical cases screened for following inclusion criteria: quarter inflammation, abnormal milk, RT >39.5°C, and a general clinical score (GCS) >3. Thirty-six out of 56 such mastitis cases (64.3%) were caused by coliform bacteria and allocated to treatment, i.e. 16 and 20 cows treated with enrofloxacin and ceftiricline, respectively. Bacteriological and clinical cures were measured 15 and 22 days later. Cure rates were better for enrofloxacin than for ceftiricline (bacteriological 81.3 vs. 65.0%; clinical 75.0 vs. 60.0%) but the differences were not significant. However, the degree and speed of improvement for the criteria MY, RT and GCS were higher in the enrofloxacin treated cows, allowing to conclude that enrofloxacin given intravenously was at least equally efficacious to an intramammary ceftiricline treatment.
Based on our observations we assume that the beneficial effects of a systemic treatment with enrofloxacin will particularly prevail in mastitis cases that are timely diagnosed and treated.
Funding: Bayer

482 (2121)

PREVALENCE OF BOVINE MASTITIS IN 20 DAIRY FARMS IN SAINT THOMAS, TEOLOYUCAN
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In order to diagnose subclinical mastitis, a California Mastitis Test (CMT) was performed on 186 dairy cows in 20 dairy farms in Saint Thomas, neighborhood Teolojucan, México state. This was done from July to October (raining season) 2001. The results were 60% positive heads, including several mastitis grades from trace up to grade 1, 2 and 3. Several microorganisms can be found nevertheless Streptococcus agalactiae and Staphylococcus aureus are the main agents of this disease. With the purpose to know their prevalence, a screen test was done on 50 (44.6%) milk samples out of the 112 that were positives to CMT with grades 2 and 3 (from 186 samples). The samples were instilled in blood agar culture and the bacteria were proceeded according to Cowan and Steel techniques in order to establish the bacterium genre. This procedure identified 41 (82%) isolates of S. aureus and 9 (18%) belonging to S. agalactiae. Once the bacterium genre was determined, a sensibility test was done through an antibiotic sensitivity plate. The antibiograms gave sensibility to bacterial agents: ampicillin, cefuraxin, captazidin, penicillin G, trimetropin, sulfametoxal and
randomly allocated to receive either routine intramammary dry cow antibiotic therapy in ipsilateral quarters in this trial. All subjects were free from intramammary infection by milk culture at dry off. The cows were Thirty (30) Holstein cows from the Elora Dairy Research Herd located outside Guelph, Ontario were enrolled off, on post-calving somatic cell counts and particle recovery in milk for seven days post-partum.

The objectives of this study were to determine the impact of OrbeSeal®, an internal teat sealant applied at dry Canada; 317300 Trans-Canada Hwy, Kirkland, PQ, H9J 2M5, Canada

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484 (1081)
SENSITIVITY AND SPECIFICITY OF THE CALIFORNIA MASTITIS TEST TO IDENTIFY THE PRESENCE OF INTRAMAMMARY INFECTIONS ON THE DAY OF DRY OFF
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The ability of the California Mastitis Test (CMT) to identify quarters containing intramammary infections (IMI) was studied in approximately 700 dairy cows from 10 Canadian Dairy Herds on the day of dry off. Bacteriological culture was used as the gold standard to identify the presence of an IMI. A positive culture for any major pathogen or greater than 10 colony forming units of Coagulase-Negative Staphylococcus was considered an IMI. Preliminary data found the sensitivity of using the CMT to identify IMI caused by all pathogens, major pathogens and Staphylococcus aureus to be 49.9%, 54.7%, and 82% respectively. The specificity of the CMT to identify IMI caused by all pathogens, major pathogens and Staphylococcus aureus was 63.1%, 64.6%, and 65% respectively. At this point in time, the CMT appears to have a role in herds in its ability to predict IMI in pre-dry cows, although there is much room for improvement.

Key word: coliform, environmental mastitis, SCC, QMS, CMT

Funding: irost

485 (1082)
THE IMPACT OF ADMINISTRATION OF AN INTERNAL TEAT SEALANT, ORBESEAL®, ON THE SOMATIC CELL COUNTS AND PARTICLES RECOVERED IN THE FIRST SEVEN DAYS POST-CALVING
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The objectives of this study were to determine the impact of OrbeSeal®, an internal teat sealant applied at dry off, on post-calving somatic cell counts and particle recovery in milk for seven days post-partum.

Thirty (30) Holstein cows from the Elora Dairy Research Herd located outside Guelph, Ontario were enrolled in this trial. All subjects were free from intramammary infection by milk culture at dry off. The cows were randomly allocated to receive either routine intramammary dry cow antibiotic therapy in ipsilateral quarters (i.e. right side or left side) or an internal teat sealant in the opposite side (i.e. right side or left side).
Quarter milk samples were taken aseptically starting the first morning milking post-calving and were repeated for seven (7) morning milkings post-partum. The somatic cell count was consistently lower in quarters that were administered the internal teat sealant than those treated with dry cow antibiotic therapy. This difference was significant on the first day. More particles were recovered from the internal teat sealant quarters than those treated with dry cow antibiotics during the first three days post-partum. After the third day post-partum, the levels of particles were the same in all of the quarters.

Use on an internal teat sealant appeared to have no detrimental effects on udder health safety when administered at dry off.

*Trade-mark of Pfizer Products Inc; Pfizer Canada Inc. licensee

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486 (2165)

STAPHYLOCOCCUS AUREUS STRAINS IN BOVINE MASTITIS ANALYZED FOR GENOTYPE BY PULSED-FIELD GEL ELECTROPHORESIS

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Purpose: Staphylococcus aureus (SA) is one of the pathogens causing intramammary infections in dairy cows, but the route of infection is not often traced. This study was conducted to identify the genotypes and phenotypes of SA strains isolated from the milk and environment of dairy farms as a means of tracing infection.

Methods: Twenty-three SA strains were found: 17 strains collected from the milk of 11 cows with clinical mastitis on five farms, 3 strains from the skin of healthy cows on two of those farms, 2 strains from milk-liners at two of the farms; 1 strain isolated from bulk-milk on a separate farm. Genotype and phenotype were determined in the 18 strains isolated from milk on six farms and in 5 strains from the environment of two farms. Production of staphylococcal enterotoxin (SE, i.e., SEA, SEB, SEC, SED) and toxic shock syndrome toxin-1 (TSST-1) was screened by reversed-passive latex agglutination respectively. Chromosomal DNA of the SA strains was prepared and their genomic DNA was fragmented with Sma1. Pulsed-field gel electrophoresis (PFGE) employed the CHEFF-DR system and four running parameters: initial pulse, 5.3 s; final pulse, 34.9 s; voltage, 6.0 V/cm; and time, 20.0 h. Phenotype was based on production of SE and TSST-1.

Results: Two phenotypes were found: one producing SE and TSST-1 (6 strains) and one not producing SE and TSST-1 (17 strains). The six strains (21.1%) producing SE and TSST-1 produced SEC but no other SE, and shared the same PFGE patterns, indicating a genetic closeness. The 17 strains (73.9%) not producing SE or TSST-1 shared similar PFGE patterns that had a negligible difference in some fragments, indicating a genetic difference from SEC- and TSST-1-producing strains. No difference occurred in the PFGE patterns of SA strains derived from milk and those from the farm environment.

Conclusion: This study had two findings. First, two phenotypes and six genotypes were in 23 SA strains, the SEC- and TSST-1-producing strains having five genotypes and the non-SEC- and non-TSST-1-producing strains a single genotype. Second, SA strains in the farm environment were not unlike those in the milk. Hence, molecular epidemiological survey of SA in the dairy environment is deemed important in preventing primary contact of the mammary gland with the pathogen, and PFGE is useful for clarifying the routes of mastitis infection.

487 (5076)

EFFECTS OF PREVENTIVE APPLICATION OF CARDUUS COMPOSITUM®, COENZYME COMPOSITUM®, LACHESIS COMPOSITUM® AND TRAUMEEL QP® ON UDDER HEALTH OF DAIRY COWS

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In the presented study, the influence of treatment with homeopathic products on the non-specific resistance mechanisms on udders of healthy cows in the periparturient period was examined.

For the study, 39 dairy cows from two herds with healthy udders were used and a double-blind experiment was performed. Therefore a medication protocol was applied. Udder health was assessed by clinical examination of the mammary gland, bacteriological status and somatic cell count (SCC) of the milk samples. Treatments were carried out on six days: drying-off (A), 270 days p.c. (B), within 12 hours after parturition (C) and on days 7, 14 and 21 p.p. (D, E and F) respectively. Treatments of the experimental group (EG) consisted of Carduus compositum® and Coenzyme compositum® (day A, B, E and F), Traumeel QP® ad. us. vet. (day C) and of Lachesis compositum® (day D). Cows of the control group (CG) received equal volumes of 0.9% NaCl. Blood samples on days A, B, D, F and on day 28 p.p. (G) were taken and concentrations of bilirubin, aspartate-aminotransferase, glutamate-dehydrogenase, total protein and beta-hydroxy-butyric acid (BHB) and differential cell count were determined.

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herd and day were pooled and examined. Milk samples were taken on days A, D, E, F and G. SCC and the bacteriological status of the samples were determined as well as lysozyme-activity in defatted milk and phagocytosis-activity of milk cells. The results of both groups were evaluated and compared. The following significant differences were noted: The value of total protein concentration in blood at day B was 75.24 g/l ± 6.91 in the EG and 69.54 g/l ± 4.82 in the CG (P < 0.05), SCC at day D was 4.84 ± 0.51 log SCC in the EG and 5.31 ± 0.71 log SCC in the CG (P < 0.01), the phagocytosis activity of milkcells at day D was 12.62% ± 6.8 in the EG and 25.05% ± 16.04 in the CG (P < 0.01) and the value of BHB at day F was 0.74 mmol/l ± 0.54 in the EG and 1.14 mmol/l ± 1.26 in the CG (P < 0.05).

In conclusion, we can say that the performed treatment with homeopathica showed a measurable influence on the non-specific resistance mechanisms of the udder and a slightly positive effect on the postparturient energy metabolism.

Funding: University of Munich

488 (2511) PHARMACOKINETICS OF MARBOFLOXACIN IN LACTATING COWS AFTER REPEATED INTRAMUSCULAR ADMINISTRATIONS AND PHARMACODYNAMICS AGAINST MASTITIS ISOLATED STRAINS
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The plasma and milk pharmacokinetics of marbofloxacin, a fluoroquinolone antibacterial compound, were evaluated in dairy cows, as well as its pharmacodynamic characteristics against mastitis isolated pathogens. Marbofloxacin was given intramuscularly as a 10% aqueous solution to dairy cows either at a single dose or at repeated doses of 2 mg/kg/sid for 3 days. Blood and milk samples were collected for the determination of the concentration of marbofloxacin and of its putative metabolites: N-desmethyl-marbofloxacin and N-oxide-marbofloxacin. Bacterial field isolates were from milk samples collected from dairy cows suspected of having an intramammary infection. After identification, the minimal inhibitory concentration (MIC) was determined against the isolated strains. The maximal marbofloxacin concentration (Cmax) observed in milk after the first administration (Cmax1stobs) was 1.024 µg/ml and the area under the curve during the first dosing interval, (AUCO-23), was 6.513 µg.h/ml. After the third administration, these parameters were slightly increased by about 20% at most. Both metabolites were detected in the milk but their concentrations were below the limit of quantification. The MIC against 90% of the population (MIC90) of Escherichia coli was 0.016 µg/ml and it was 0.229 µg/ml against Staphylococcus aureus. The following surrogate clinical outcome markers were obtained against E. coli strains: a Cmax / MIC ratio of 67 and an AUC / MIC ratio of 407 h. Hence, a possible efficacy of marbofloxacin in the treatment of E. coli induced mastitis could be expected as the endpoints of 10 and 250 h respectively are reached.

489 (2617) DIFFERENTIAL CELL COUNT AND INTERDEPENDENCE OF UDDER QUARTERS
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Goal of the study: Indicators for the interdependence of udder quarters within one cow, i.e. compensation of milk yield after loss of one quarter, have been described since many years. Still, many studies are based on the hypothesis of quarter independence when using within cow controls. The aim of this study was to evaluate the interdependence of udder quarters by the means of microscopic and flow cytometric cell differentiation in milk.

Material and methods: The physiological reference (group A) comprised 52 quarter milk samples of 13 cows which had been monitored during their entire lactation and did not exhibit even subclinical mastitis. The samples of 9 cows with a somatic cell count (SCC) of more than 100000/ml but less than 400000/ml in at least one quarter were divided into two groups: SCC <100000/ml (group B1) and SCC >100000/ml but <400000/ml (group B2). Another 17 cows with SCC >400000/ml in at least one quarter were included and their milk classified as SCC <100000/ml (group C1), SCC >100000/ml but <400000/ml (group C2) and SCC >400000/ml (group C3).

For the microscopic cell differentiation, the cells were isolated and washed three times before being spread on a slide by centrifugal force. The smears were stained with the system Hemacolor (Merck). For the microscopic cell differentiation, the cells were isolated and washed three times before being spread on a slide by centrifugal force. The smears were stained with the system Hemacolor (Merck). The same cell suspension was also incubated with a monoclonal antibody (mab) against Bo116, a structure on the surface of bovine polymorphonuclear neutrophil granulocytes (PMN) and with the mab CC8 against bovine CD4.

Results: The percentages of lymphocytes (25%) and CD4 positive cells (20%) were significantly (p<0.0001) higher in group A than in all other groups. Similar results were found for macrophages with the exception of group B1. The values for PMN and Bo116 were significantly lower (p<0.0001) in group A (34% and 5%, respectively) than in all other groups, except group B1.

Implications: These data indicate that the infection and inflammation of one quarter do influence the differential cell count of the other quarters within the same udder. Therefore, the quarters of one mammary gland cannot be regarded as four isolated units since they communicate with each other, i.e. they are
interdependent.

490 (2619)
A CASE STUDY ON THE EXCRETION OF STAPHYLOCOCCUS AUREUS IN MILK OVER A 30-DAY PERIOD
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Goal of the study: Staphylococcus (S.) aureus is one of the most important mastitis pathogens. It is assumed this organism is shed only intermittently and therefore repeated sampling with a weekly interval is recommended. However, little is known about the S. aureus concentration in milk and its relation to the somatic cell count (SCC) after the infection itself and the inflammation are established. Hence, a study was designed to investigate the pattern of S. aureus excretion. Secondly, the diagnostic benefit of culturing 50 µl vs. 10 µl of milk was evaluated.

Material and methods: Quarter foremilk samples of 20 cows (80 quarters) infected with S. aureus in at least one quarter were obtained daily at morning milking over a period of 30 days. The somatic cell count (SCC) was determined by Fossomatic. For the microbiological evaluation, samples were spread twice on blood agar plate halves using 10 µl and 50 µl of milk. To distinguish S. aureus from haemolysing coagulase negative staphylococci (CNS), colonies were tested for clumping factor, hyaluronidase and coagulase.

Results: In 23 quarters S. aureus was shed daily and 35 quarters were bacteriologically negative throughout the sampling period. In the remaining 22 quarters, other pathogens were diagnosed and therefore the quarters excluded from further analysis. None of the sampled quarters showed an intermittent S. aureus excretion pattern. Statistical analysis of the 23 S. aureus quarters revealed no correlation between bacteria concentration and SCC. Overall, both parameters stayed on the same level throughout the sampling period. Regarding microbiological findings, 95% of the all 1145 S. aureus diagnoses could be based on the examination of 10 µl. On plates containing 50 µl, growth was often too dense to allow subcultivation of single colonies.

Implications: It was concluded that there is no relation between the S. aureus concentration in milk and the cellular reaction of udder quarters. This case study also indicates that at least in chronically infected quarters S. aureus can be diagnosed regularly. Accordingly, one single sampling might be enough to conduct a herd screening, whereas a sanitation program should be based on three samplings in a weekly interval. Spreading 10 µl on half a blood agar plate is preferable to using 50 µl. If a higher diagnostic sensitivity is required, two or three parallel analyses of 10 µl should be conducted.

491 (827)
THE CONCENTRATIONS OF HUMAN AND BOVINE LACTOFERRIN IN THE MILK OF RHLF-TRANSGENIC COWS IN EXPERIMENTALLY INDUCED E.COLI- MASTITIS
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Lactoferrin is an iron-binding glycoprotein synthesized and released by mucosal epithelial cells and neutrophils. It serves as a part of nonspecific defence system and is bacteriostatic for a variety of microorganisms like coliform bacteria.

We challenged seven transgenic first-calving Holstein-Friesian cows (generated by Pharming Group NV, Holland) expressing recombinant human lactoferrin (rhLF) in the milk with Escherichia coli bacteria via the teatcanal and measured response, clinical signs as well as milk and blood values. By ELISA rhLF and bovine lactoferrin (bLF) were quantified separately. Concentration of rhLF was constant (about 2.65 g/l); bLF concentrations ranged from 0.07 to 0.21 g/l (mean 0.11 g/l).

It may be possible to improve the defence mechanism in the dairy cow mammary gland by use of rhLF in the milk or by using exogenic bLF to support antimicrobial treatment and eventually reduce the use of antibiotics in therapy of coliform mastitis.

492 (1335)
THE STUDY ON CORRESPONDENCE BETWEEN THE RESULTS OF COWS AND BULK TANK CULTURES REGARDING THE STREPTOCOCCUS AGALACTIAE IN TWO HERDS IN IRAN
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Introduction: Due to the obligate nature of Streptococcus agalactiae (S. ag.), its presence in bulk tank milk (BTM) is the exclusive result of shedding of bacteria from infected quarters. Consequently, BTM has a very high specificity, but with use of traditional milk culture techniques, a low sensitivity for identifying S. ag. at the herd level. The objective of this study was to determine the association of the bulk tank milk culture results with the results of the corresponding cow milk cultures regarding the S. ag. in two dairy herds in Iran.

Material and methods: The study was conducted in two dairy herds with 852 (Herd A) and 252 (Herd B) lactating cows. Composite milk samples were aseptically collected from all lactating cows in both herds and
BTM samples were collected bi-monthly for the periods of 1 and 3 ½ months in herds A and B respectively, including on the days of herds’ cultures. BTSCC was measured using direct microscopic counting method and microbiological procedures were conducted in accordance with National Mastitis Council (NMC) standards.

Results: Based on the total herds’ cultures, S. ag. was isolated from 39 (4.58%) and 77 (30.55%) cows in the herds A and B respectively. On the days of herds’ cultures, 8000 cfu/ml of S. ag. were detected from the bulk tank milk of the herd B (with higher intra-herd prevalence) versus the negative results for the herd A (with lower intra-herd prevalence) despite the presence of at least 39 infected cows in this herd. The averages of BTSCC were 277,165 and 610,521 cells/ml in herds A and B, respectively, which also indicated the higher prevalence of mastitis in herd B. In addition, during the periods of bulk tank milk analysis, S. ag. was isolated from 1 out of 2 (50%) and 6 out of 7 (85.71%) bi-monthly samples in herds A and B, respectively.

Conclusions: The results of this study demonstrate that the function of difference in intra-herd prevalence, and variation in the rate of bacterial shedding, are probably responsible for discrepancy between cows and bulk tank milk culture results in some conditions. In addition, the absence of S. ag. in a single bulk milk sample does not mean that the organism is not present in the herd. Therefore, establishing a bulk tank milk profile through multiple sampling is imperative to make such a decision. New culture methods, using selective media and large inocula, are also needed to improve the sensitivity of bulk tank milk culture.

Funding: University of Tehran

493 (2116)
BACTERICIDAL ACTIVITY OF PENICILLIN G AND CONDENSED TANNINS AGAINST BOVINE MASTITIS PATHOGENS
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The following study was conducted to determine if the condensed tannins, catechin hydrate ( (+) trans-3, 3’, 4’, 5, 7-pentahydroxyflavane, CH) and quebracho tannins (4-6 or 4-8 linked subunits of 5-deoxyflavan-3-ol fisetinidol, QT) enhances the bactericidal activity of penicillin G against Staphylococcus aureus, Streptococcus uberis, and Klebsiella oxytoca that were isolated from mastitic mammary gland quarters. Each bacterial species was incubated with various concentrations of CH or QT and penicillin G in a microdilution growth inhibition assay. Synergistic antibacterial activity against the bacterial isolates was observed. An increase in bactericidal activity as a function of increasing CH or QT concentration was observed in both the presence and absence of penicillin G. A CH concentration of 3.0 mg/ml reduced the minimum inhibitory concentration (MIC) of penicillin G against the S. aureus isolate by 1/2x. A combination of 0.5 mg/ml CH and penicillin G at 1/2x MIC was bactericidal for the S. uberis isolate. Catechin hydrate alone was bactericidal against S. aureus at a concentration of 4.0 mg/ml and against S. uberis at a concentration of 2 mg/ml. The K. oxytoca isolate appeared to be resistant to the combined effect of CH and penicillin G, or 5.0 mg/ml CH alone. Quebracho tannin alone was bactericidal against S. aureus at a concentration of 0.25 mg/ml. A combination of 0.5 mg/ml QT and penicillin G at 1/4x MIC, and a concentration of 1.0 mg/ml QT alone were bactericidal for the S. uberis isolate. A QT concentration of 0.50 and 2.0 mg/ml reduced the MIC of penicillin G against K. oxytoca by 1/2x and 1/8x. However, 5.0 mg/ml QT alone was not bactericidal against K. oxytoca. The condensed tannins, CH and QT, may potentiate the bactericidal activity of penicillin G against Gram-positive and Gram-negative bacterial pathogens that infect the mammary gland.

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494 (2364)
MICRO ORGANISM ISOLATION PREVALENCE IN BOVINE MILK SAMPLE OF THE PROVINCE OF BRESCIA AND IN VITRO ANTIBIOTIC SENSITIVITY
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This paper describes microorganisms isolation prevalence from 192105 milk samples collected from udder with or without mastitis, during the period from January 2002 and October 2003. Antibiogram results to evaluated in vitro sensitivity of different isolated bacteria are also reported. Micro organisms isolation and identification were conducted following two different diagnostic protocols: bacteriological reference method using blood agar media, for micro organism isolation from single quarter samples, collected from udders without a specific diagnostic hypothesis; bacteriological method using selective media (i.e.: T.K.T. for Streptococcus agalactiae, Baird - Parker supplemented with RPF for Staphylococcus aureus), for microorganism detection from composite milk sample collected from udder without clinical signs.

Results are reported as follow: 8155 single quarter samples collected from udders without a specific diagnostic hypothesis were analysed; 45.64% samples were found positive, 31.43% without bacterial grow and 1.73% without output for over contamination. In positive samples: 4.93% Streptococcus agalactiae, 3.56% Streptococcus uberis, 5.79 different streptococi, 26.13% Gen. Enterococcus, 17.26% coagulase positive Staphylococci, 16.40% coagulase negative Staphylococci, 12.38% Fam. Enterobacteriaceae, 2.01% Gen Bacillus, 1.5% yeast 0.94% Gen Proteotheca and 9.06% different various micro organisms.

99085 composite milk samples were analysed for Streptococcus agalactiae detection, collected for an
intramammary infection control programme. 16.71% samples were found positive, 82.76% negative and 0.54% without output for over contamination by Streptococcus uberis or Gen. Proteus. 84685 composite milk samples were analysed for Staphylococcus aureus detection, collected for an intramammary infection control programme; 16.2% samples were found positive, 82.07% negative and 1.73% without output for over contamination by mould.

Positive results were also ordered by isolation period to evaluate seasonal difference. Kirby-Bauer antibiotic sensitivity test were performed for isolated microorganisms (NCCLS procedure) to evaluate in vitro antibiotic activity.

Funding: Istituto Zooprofylattico Sperimentale della Lombardia ed Emilia Romagna -Brescia- Italy

495 (2591)
EPIDEMIOLOGY OF ACUTE BOVINE ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS MASTITIS
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The purpose of the study was to describe and compare certain epidemiologic features of acute mastitis caused by Escherichia coli or Staphylococcus aureus.

During a one-year field study in southeastern Norway, data were recorded and quarter milk samples collected for bacteriological examination from 394 cases of clinical mastitis. The current study includes 63 cases caused by E. coli in 43 different dairy herds and 174 cases caused by S. aureus in 126 different herds. All cases were examined and treated by veterinary surgeons.

Of the cases caused by E. coli, 25% occurred within 5 days postpartum, while 36% of the S. aureus cases occurred in that period. Thirty-eight percent of E. coli cases and 24% of S. aureus cases were treated between days 6 and 60 postpartum. Only small differences between the relative proportions of E. coli and S. aureus cases were observed during the remaining part of lactation. Cows of parity 3 or more were at a greater risk of suffering an episode of E. coli mastitis than were younger cows (odds ratio = 2.2); the corresponding OR for cows with S. aureus mastitis was 1.5. The relative proportion of peripartal cases of E. coli mastitis was greater among cows of parity 1 and 2 as compared with older cows. For cases of S. aureus mastitis, no relationship was observed between age and the time of occurrence in the lactation period. The monthly distribution of calvings for cases and for all cows in the herds in which cases occurred revealed a tendency for cows that calved in summer months (July-September) to be at a greater risk of peripartum E. coli mastitis as compared with cows that calved between October and June. Conversely, there was a tendency for cows that calved in summer to be at a lower risk of peripartal S. aureus mastitis than those that calved at other times of the year. Cases were grouped in four categories by severity of clinical signs. Twenty-seven percent of cows with E. coli mastitis and 12% of cows with S. aureus mastitis showed marked or severe systemic signs. Peripartal cases of E. coli or S. aureus mastitis were not associated with more severe clinical signs than cases occurring later in the lactation period. For both E. coli and S. aureus cases, there was no relationship between parity of cows and severity of clinical signs.

The study revealed differences in the epidemiology of E. coli mastitis and S. aureus mastitis, demonstrating that bacteriology should be included in precise studies of bovine mastitis.

496 (3406)
EFFICACY OF A NEW CEFQUINOME DRY COW FORMULATION IN A CONTROLLED, RANDOMISED AND PARTIALLY BLINDED FIELD STUDY
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Purpose: Cefquinome is broad spectrum cephalosporin active against aerobe, anaerobe, Gram-positive and -negative bacteria. Antibiotic dry cow treatment is used for the elimination of existing infections at drying-off and for the prevention of new infections of the dry period. In a multicentre field study a new cefquinome formulation (Cobactan® DC) was compared to Orbenor® Hors Lactation.

Methods: A total of 263 lactating cows, 35 and 70 days prior to the calculated calving date, not pre-treated in the 30 last days and with a positive California Mastitis Test in at least one quarter were enrolled. They were selected in 68 farms with a geometric mean bulk Somatic Cell Count of 203 000 cells/mL (68 000 - 463 000) in 4 European countries. The cows were randomly allocated to the Cobactan® or the Orbenor® group and treated after the last milking (150 mg cefquinome and 600 mg cloxacillin respectively). Quarter milk samples were taken aseptically on day -2, day -1, day 0 (treatment), day of calving and 5-7 days after calving. A quarter was regarded bacteriologically cured when both postcalving samples showed no bacterial growth or when one or both postcalving samples showed bacterial growth of a species different from that isolated pretreatment. New infections were defined as the appearance of a new bacterial species different from that isolated at drying-off or by proving bacterial growth in postcalving samples which were not infected at drying-off or in the case of clinical mastitis developing between drying-off and 5-7 days postcalving.

Results: The overall bacteriological cure rate was equivalent between the treatment groups (significant therapeutic equivalence test). For staphylococci it was 83.1% in the Cobactan® group and 81.4% in the Orbenor® group. All S. uberis infections cured in both groups but from a limited number of cases. No difference was observed between the 2 groups for the overall new infection rate. However less infections due
to E. coli or other Enterobacteriaceae were recorded in the Cobactan® group than in the Orbenor® group (8 vs 20 cases).

Conclusions: In this multicentre field study the new cefquinome dry cow formulation was effective in both the elimination of bacterial infections in the mammary gland at drying-off and in the prevention of new infections during the dry period.

500 (1377)
THE EFFECT OF POLLUTION ON TRACE METAL ACCUMULATION IN CALVES (ASTURIAS, NORTHERN SPAIN)
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Asturias (North Spain) is a region with a large industrial and mining area located at its centre. The rest of the territory is especially rural, and cattle production is the most important form of agriculture. Industrial and agricultural development has been responsible for the diffusion of metals in the environment, these metals are accumulated in soils and edible plants, and when animals are fed with these plants, they accumulate high levels of toxic metals in their organism. Monitoring levels of mineral concentrations in animals is important for assessing the effect on animal health, and trace mineral imbalances are also an important part of production efficiency. For these reasons, Asturias may deserve special attention with regard to possible metal contamination in livestock. The aim of this study is evaluate the metal (Cu, Zn, Fe and Mn) accumulation in calves from the industrialized area of Asturias and to evaluate if there are differences in metal accumulation depending on the area of raising.

Samples of liver, kidney, muscle and blood of 78 animals from the industrialized area and 92 from the rural area were collected. Samples were acid digested and were analysed by flame using atomic absorption spectrophotometry (AAS).

There was no evidence of toxic accumulation of trace elements in Asturian calves. Calves from the industrial area showed significantly lower copper, zinc and manganese residues in liver. Zinc, iron and manganese were similar to those reported in other studies. However, copper levels in calves from the industrialized area of Asturias were generally low and up to 41% of animals raised there might be at risk from copper deficiency.

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