T158 Consumer perception regarding beef quality and food safety. Maribel Ruiz-Leon, Karina Leon-Lucio, Gilberto Aranda-Osorio*, and Agustin Ruiz-Flores, *Universidad Autonoma Chapingo, Chapingo, Texcoco, Mexico.*

The aim of this study was to evaluate consumers' perception and attitude regarding beef quality and food safety and the characteristics that are taken into account for purchasing, considering socio-economic factors such as income and education level. A survey integrated by 70 questions, grouped into 6 sections, was applied to 347 administrative and academic staff belonging to the University of Chapingo. The sample population was divided into 4 monthly income levels (strata): 1) Low (less than 7,000 pesos), 2) Medium (from 7,000 to 14,000 pesos), 3) High (from 14,000 to 21,000 pesos) and 4) Very high (more than 21,000 pesos). USD exchange ratio: 1USD = 13.50 pesos. Data were processed by discriminatory canonical analysis to identify the main factors that affect beef consumption; after that, association tests between the income level and beef consumption variables were performed using the ANOVA and the statistic Chi-squared of SPSS. The results showed dependence ($P < 0.05$) between income and education level to: preference for purchasing place (butcheries > supermarkets > street markets) and meat type (poultry > beef > pork) according to the conservation method (chilled > not chilled > frozen); perception and preference for TIF (Federal Inspection Type) seal (42%); preference for the amount of fat (60%, low) and beef color (80%, bright red); knowledge about the use of clenbuterol on finishing cattle (90%) and its negative effect on consumer health (62%); willingness for purchasing beef-free of clenbuterol (100%); knowledge about the concept of food safety (35%); perception of the importance of safe beef and its relation to human health (32%). In conclusion, the awareness about beef safety positively grows with the income and education levels of the consumers, encouraging them to search for safe markets, which ensure beef safety and quality.

Key Words: beef quality, food safety, consumer characteristic

T159 Levels of aflatoxin M_1 in dairy products from Londrina supermarkets and its estimated daily intake. Joice Sifuentes dos Santos*, Ana Beatriz C. Ribeiro, Vanessa R. França, Shiguedy Katto, and Elsa Helena W. Santana, *University North of Paraná, Londrina, Paraná, Brazil.*

Aflatoxins are fungi secondary metabolites that contaminate cereals and other products of vegetable origin. Aflatoxin B_1 (AFB_1) is the most common and the most toxic aflatoxin. After the ingestion of AFB_1 contaminated feeds, a part is degraded in the rumen, resulting in the formation of aflatoxicol. The remaining fraction is absorbed in the digestive tract by passive diffusion and is hydroxylated in the liver to aflatoxin M_1 (AFM_1). Circulating AFM_1 can be excreted in the urine or appear in milk. AFM_1 excretion is also observed in human milk. AFM_1 was originally classified as a Group 2B – possibly carcinogenic to humans, in 1993, but subsequent evidence of its cytotoxic, genotoxic and carcinogenic effects led to a new categorization of AFM_1 as Group 1. The occurrence of aflatoxin M_1 (AFM_1) was evaluated in 42 milk (pasteurized, ultra-high treated (UHT) milk and milk powder) randomly collected in July 2014 from supermarkets in Londrina, Paraná State, Brazil and this rate of occurrence was used to estimate AFM_1 exposure. AFM_1 determination was carried out by ELISA. AFM_1 was detected in 100% samples, with levels ranging from 0.01 to 0.81 µg/kg, and a mean of 0.13 µg/kg. Differences were observed in AFM_1 levels in milk powder samples (0.61 µg/kg) compared with pasteurized (0.02 µg/kg) and UHT milk (0.04 µg/kg; $P < 0.05$). None of the samples presented AFM_1 above the maximum permitted level by Brazilian Legislation (0.5 µg/kg for fluid milk and 5 µg/kg for milk powder). Determination of the exposure degree is one of the most important parameters to assess risk from chemical compounds. The Estimated Daily Intake (EDI) of AFM_1 from fluid milk and milk powder was obtained using the amount of food consumed and the corresponding mean concentrations of AFM_1 detected in each food group, taking into account the mean body weight of the age groups. The estimated daily intake (EDI) of AFM_1 was evaluated, and the average intake was 0.468 ng/kg body weight (BW) for adolescents, 0.384 ng/kg BW for adults and 0.559 ng/kg BW for the elderly, values that pose a toxicological risk to the population investigated.

Key Words: mycotoxin, dairy product, ELISA

T160 Occurrence of aflatoxin M_1 and somatic cell count in milk from farms in São Paulo, Brazil. AF Rosa[1], MS Miranda[1], JRP Arcaro[1], R. Braghini[2], E. Pinatti[3], and CR Pozzi*[1], *[1]Instituto de Zootecnia, Nova Odessa, São Paulo, Brazil, [2]Instituto de Ciências Biomédicas, São Paulo, São Paulo, Brazil, [3]Instituto de Economia Agrícola, São Paulo, São Paulo, Brazil.*

Milk needs to be of good quality and a low somatic cell count (SCC) and absence of contaminants such as aflatoxin M_1 (AFM_1) are important factors in the assessment of the health status of herds. The objective of this study was to evaluate the presence of aflatoxin M_1 and SCC in milk and to determine the correlation between aflatoxin M_1 and SCC. Fifteen lactating cows were monitored at intervals of 15 d over a period of 45 d (n = 4) on 9 dairy farms in the state of São Paulo (n = 540). Milk samples were collected from the glass collecting jars (n = 15) and refrigeration tanks (n = 38). Aflatoxin M_1 was determined on immunoaffinity columns using separation and quantification by high-performance liquid chromatography (HPLC). Somatic cell counting was performed by flow cytometry in a Somacount 300. Data were analyzed using a one-way ANOVA with Tukey's test ($P \leq 0.05$). Pearson correlation test was used between AFM_1 and CCS. Aflatoxin M_1 was detected in 12% (n = 64) of the milk samples collected from the glass bottles. Mean contamination of the samples ranged from 0.03 ± 0.09 to 0.746 ± 1.85 µg/kg, with a maximum level of 9.83 µg/kg. Of the 38 milk samples obtained from the refrigeration tanks, 16% (n = 6) were contaminated with aflatoxin M_1, with levels ranging from 0.44 to 2.65 µg/kg; 83% (n = 4.98) of these samples contained levels that exceeded the limit permitted by the Brazilian legislation (0.5 µg/kg). Mean SCC in the glass collecting bottles ranged from 283.78 (±1,302.12) to 1,124.58 (±1,844.9) x 1,000 cells/mL. Mean SCC in the refrigeration tanks ranged from 452.75 (±220.7) to 2,057.75 (±1,233) x 1,000 cells/mL. All farms had tanks with SCC above the limit permitted by the current Brazilian legislation (500 × 1,000 cells/mL). There was no correlation between the detection of aflatoxin M_1 and SCC in milk samples from the glass collecting jars or refrigeration tanks. The presence of aflatoxin M_1 and high SCC indicate deficiency in the sanitation management of the farms and highlight the need for public policies to improve these patterns.

Key Words: aflatoxin M_1, somatic cell count, milk

T161 Risk assessment of seven toxic elements residues in raw milk in China. XueYin Qu[1,2], Nan Zheng[1,2], JiaQi Wang*[1,2], XueWei Zhou[1,2], and SongLi Li[1,2], *[1]Ministry of Agriculture-Laboratory of*

Quality & Safety Risk Assessment for Dairy Products (Beijing), Beijing, China, [2]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.

The pollution of toxic elements has been serious since the rapid development of industrialization in China. There were no data about toxic element levels in raw milk in China. The object of this study was to survey the levels of 7 toxic elements residues in raw milk in China and assess the potential health risk of those residues. The 178 raw milk samples were collected from 8 main milk-producing provinces and from 3 types of milk stations in China, and were analyzed for arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr), mercury (Hg), aluminum (Al) and nickel (Ni) using inductively coupled plasma-mass spectrometry (ICP-MS). Al, Pb, Hg, Ni, Cr, As were detected in 47.8% 29.2%, 28.1%,23.6%, 12.4% and 9.0% of total milk samples, respectively, and Cd was not detected in any samples. The levels of detected toxic elements were all below China's regulated limits of As ≤0.1 mg/kg, Pb ≤0.05 mg/kg, Cr ≤0.3 mg/kg, Hg ≤0.01 mg/kg. The residue levels of the samples from the processing plants were lower than that from the large-scale farms and small farm cooperatives by the results of Nemerow pollution index analysis method and this could be related with the different manufacturing practices. The regional difference analysis results indicate that the raw milk samples from heavy industry provinces have relative high residue levels. The need for further attention to the raw milk of heavy industry regions is crucial. In all detected samples, the risks of the concentrations were far below the reference values. The HQ analysis showed that the residues of As, Pb, Hg, Cr, Al and Ni in the raw milk samples were not presenting potential risk to Chinese adult, if the daily intake was 300 mL.

Key Words: raw milk, toxic elements, China

T162 Concentration of 22 elements in milk, feed and water of dairy cow, goat, and buffalo from different regions of China. Xuewei Zhou[1,2], Xueyin Qu[1,3], Nan Zheng[1,3], Fadi Li[2], and Jiaqi Wang*[1,3], *[1]Ministry of Agriculture Laboratory of Quality & Safety Risk Assessment for Dairy Products (Beijing), Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]College of Animal Science and Technology, Gansu Agriculture University, Lanzhou, Gansu, China, [3]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Milk is the most diversified natural food contained more than 20 trace elements that are necessary for human health. However, metals such as Pb, Cr, Hg, As and so on, may contaminate the environment and thus the feed and water, passing into milk and causing health problems. In our study, the content of 22 elements, including Fe, Mn, Cu, Zn, Co, Al, V, Cr, Ni, Ga, As, Se, Rb, Sr, Ag, Cd, Cs, Ba, Hg, Tl, Pb and U, in cow milk (40), goat milk (40) and buffalo milk (20) in China were analyzed. These 22 elements in their corresponding feed and water were also examined using inductively coupled plasma-mass spectrometry (ICP-MS) after microwave-assisted acid digestion. Significant differences ($P < 0.05$) with mean values were analyzed by Tukey's HSD test in SPSS 17.0. The mean spiked recovery of analytical method was 70.96–113.41% in milk, 71.2–114.8% in feed, and 86.50–123.30% in water. In milk, the mean content of Rb and Zn showed high levels (>1000μg/L), followed by Al, Fe, Sr and Ba (100–1000 μg/L), and then Mn, Cu, Se and Cs (10~100μg/L). The mean values of V, Cr, Co, Ni, Ga, As, Ag, Cd, Hg, Tl, Pb and U were less than 10 μg/L. Co, Rb, Sr, Cs and Tl levels showed significant difference ($P < 0.05$) among cow, goat and buffalo milk. The content of Rb, Sr, Pb, Cs, As, Ni, Tl in drinking water were significant ($P < 0.05$) correlated with those in milk. However, elements in feed were not ($P > 0.05$) related to milk. It suggested that elements in water might contribute the content of elements in milk. The levels of As, Cd, Hg and Pb were under the MRL of China and did not pose threat to consumers.

Key Words: element, milk, ICP-MS

T163 Effect of HTST and UHT processing on the stability of cephalosporin residues in milk. Meixia Chen[1,2], Nan Zheng[1,2], Fang Wen[1,2], Hui Wang[1,2], Songli Li[1], and Jiaqi Wang*[1,2], *[1]Ministry of Agriculture Laboratory of Quality & Safety Risk Assessment for Dairy Products (Beijing), Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]Ministry of Agriculture-Milk and Dairy Product Inspection Center, Beijing, China, [3]State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Presence of antibiotics in raw milk threatens human health and dairy production. Heat treatments are expected to inactivate various pathogens or reduce the amount of other undesired substances such as antibiotics before milk reach consumers. As a common part of cephalosporins' molecular structure, β-lactam ring makes cephalosporins susceptive to chemical reaction. Hence, cephalosporins are very likely to experience decrease once heated. To date, few information about cephalosporins' stability during conventional dairy process such as high temperature short time pasteurization (HTST) and ultrahigh temperature process (UHT) has been reported. This study was designed to study the effects of HTST and UHT on cephalosporins. So heat treatment were performed on raw milk samples spiked with 5 cephalosporins—cefoperazone (PER), cefquinome (QUI), cephapirin (PIR), ceftiofur (TIO), cefazolin (ZOL)—on the level of maximum residues limits (MRLs). Of these treatments, 65°C/15s was used for preheating raw milk, 20M Pa for homogenization, 75°C/15s for HTST and 140°C/4s for UHT. Concentration of 5 cephalosporins in milk was determined by UPLC-MS/MS simultaneously before and after heat treatment. Recovery test was conducted to validate the analytical methods used in this study for determination of 5 cephalosporins in our lab. SAS 9.2 statistical software package were applied to carry out the statistical analysis. Data of recovery ranges from 85.8% to 99.8% with RSD lower than 10%, which demonstrates good recovery was obtained. Degradation percentages of cephalosporins obtained after HTST is as following: 2.6% for ZOL, 8.6% for PIR, 12.2% for QUI and 12.3% for TIO with the highest 20.5% for PER. Whereas UHT process showed higher reduction level of cephalosporins in all cases. As is shown, cephalosporins' loss is 15.7% for PIR, 36.3% for PER, 43.9% for QUI, 50.0% for TIO with the highest 77.4% for ZOL after UHT. It is noteworthy that degradation results obtained in our work is higher than the previous data predicted by M. Roca (2011) using the prediction model based on the kinetic equation. In addition, the effect of HTST and UHT displays significant difference ($P < 0.01$). In conclusion, cephalosporins studied in this work is resistant to HTST but is unstable in UHT.

Key Words: cephalosporins, HTST, UHT

T164 Toxins in milk of cows fed with transgenic maize. Geraldo Neto Balieiro*[1,3], Keila Maria Roncato Duarte[2], Roberto Botelho Ferraz Branco[1], and Acyr Vanderley de Paula Freitas[1], *[1]São Paulo State Agency Agribusiness Technology, Ribeirão Preto, São Paulo, Brazil, [2]São Paulo State Agency Agribusiness Technology, Nova Odessa, São Paulo, Brazil, [3]Research Supported by FAPESP, São Paulo, Brazil.*

The goal of this study was to evaluate the presence Cry toxin in milk of cows feeding transgenic maize hybrids. Twenty-four Jersey cows were allotted into 2 groups: control (n = 12), feeding conventional diet, or test, consuming grain and silage from maize with *cry* gene from *Bacillus thuringiensis* (n = 12). A paired *t*-test was used to determine whether there were significant differences between the 2 treatments. Were used 0.5 g of maize leaves transgenic lyophilized, macerated in liquid nitrogen, resuspended in 1 mL of methanol (80%) and diluted with 4 mL of PBS buffer as immunoassay antigen. Two female New Zealand rabbits were used to produce polyclonal antibodies against Cry1A105, Cry2Ab2, Cry1F, Cry1Ab and VP3Aa20 toxins from DKB 390 VT Pro II, AG 8088 Pro II, Biomatrix 2B655 Hx, Syngenta Impacto TL TG and Syngenta 7205 Viptera, respectively, to be used as a screening for maize-derived food products, by plate-trapped antigen (PTA)-ELISA. Immunogen was prepared bioconjugating Cry toxin to carbodiimides. Immunizations were performed each 15 d. At the end of 5 immunizations the rabbits were bled and sera were kept at –80°C. Antibodies were purified in immunoaffinity columns between 50 and 100 kDa. Milk samples, collected 3 time each 14 d during 56 d, were frozen and lyophilized. Antigen (100 mL) were placed into micro wells in triplicates and incubated 1 h at 37°C. Blocking were done using 200 mL of PBS buffer added to 1% BSA for 1 h 37°C. Plates were dried and 100 mL of anti-sera dilution (1:500) were placed, for 1 h at 37°C. Plates were washed (PBS) and anti-rabbit conjugated HRP sera dilution (1:1000) was added. After 1 h at 37°C, plates were washed (PBS-T-G) and PNPP (*p*-nitrophenyl phosphate) revealed, at 450 nm. The presence Cry toxin analysis did not differ between milk of cows fed by transgenic maize hybrids and its nonbiotech counterpart. Results showed that the milk system production using silage and ration with transgenic maize hybrids that contains cry gene is safe and the antibodies produced through leaves as antigen are highly sensitive and specific to Cry toxin and can be used on an enzyme immunosorbent with low cost, high sensibility and specificity.

Key Words: GMO, food security, plate-trapped antigen (PTA)-ELISA

T165 Aflatoxin B_1 and aflatoxin M_1 induced cytotoxicity and DNA damage in differentiated and undifferentiated Caco-2 cells. Jie Zhang[1,2], Nan Zheng[1,3], Fadi Li[2], Songli Li[1,3], and Jiaqi Wang*[1,3], *[1]Ministry of Agriculture Laboratory of Quality & Safety Risk Assessment for Dairy Products (Beijing), Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]College of Animal Science and Technology, Gansu Agricultural University, Lanzhou, China, [3]State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Aflatoxin B1 (AFB_1) and aflatoxin M_1 (AFM_1) are natural mycotoxins that frequently found in food and feed. Numerous reports have indicated that these toxins pose serious risks to human and animal health. However, there are few data in the literature regarding the impairment of AFB_1 and AFM_1 on intestine. The present study therefore was undertaken to investigate the cytotoxic effect and DNA damage of these toxins on human colon carcinoma cell line Caco-2, especially the differentiated cells that resemble mature small intestinal enterocytes. Both undifferentiated (UC) and differentiated (DC) cell were treated with AFB_1 and AFM_1 at various concentrations for 24, 48 and 72 h. Cell viability, lactate dehydrogenase (LDH) release and reactive oxygen species (ROS) production were determined, and DNA damage was accessed by comet assay. Statistical analysis of data was carried out using SAS9.2, statistical software package. Data showed that AFB_1 and AFM_1 inhibited UC and DC cell growth and increased the LDH release in a time- and dose-dependent manner. Besides, AFB_1 treatment resulted in an evident increase in cytotoxicity over AFM_1 at the high dosage ($P < 0.05$). Moreover, DC were more sensitive to toxins compared with UC ($P < 0.05$), particularly after exposure of 72 h at the dose of 1 μg/mL, as indicated from the lower cell viability(48% vs. 67%, AFB_1 treatment) and the higher LDH release (118% vs. 186%, AFM_1 treatment; 142% vs. 194%, AFB_1 treatment). Marked impacts in the genetic damage were observed after treatment with 2 toxins on UC and DC, even higher than those of H_2O_2 treatment (positive control). Compared with UC, DC also had more DNA damage ($P < 0.05$), which might due to the alteration of cells during differentiation. All these cytotoxic effects might associate with the strong intracellular ROS generation in the presence of toxins. The present study provided the first experimental evidence of the in vitro DNA damage of DC induced by AFB1 and AFM1.

Key Words: aflatoxin B_1, aflatoxin M_1, DNA damage

T166 Interaction of aflatoxin M1, ochratoxin A, zearalenone, and α-zearalenol combinations on Caco-2 cells. Yanan Gao[1,2], Nan Zheng[1,2], Songli Li[1,2], Yangdong Zhang[1,2], and Jiaqi Wang*[1,2], *[1]Ministry of Agriculture Laboratory of Quality & Safety Risk Assessment for Dairy Products (Beijing), Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

It is common to find the co-occurrence of aflatoxin B_1 (AFB_1), ochratoxin A (OTA), zearalenone (ZEA) in feed. These mycotoxins can be metabolized or transferred to raw milk in the forms of Aflatoxin M1 (AFM_1), ochratoxin A (OTA), ZEA, and α-zearalenol (α-ZOL), which are stable in processing of dairy products. The aim of the present study is to investigate the cytotoxicity of combined mycotoxins of AFM1, OTA, ZEA, and α-ZOL on the human colon adenocarcinoma cell lines (Caco-2) by using the tetrazolium salt (MTT) assay and the isobologram analysis. Statistical analysis of data was carried out using SAS9.2 statistical software package. Our results demonstrated the significant cytotoxic effects ($P < 0.05$) of the 2-toxin, 3-toxin and 4-toxin combination on Caco-2 cells in a time- and concentration-dependent manner. The results from MTT assay demonstrated that AFM_1 had the similar cytotoxicity as OTA (IC_{50} = 4.10 μM) upon exposure for 72 h, which was much higher than that of ZEA and α-ZOL. The cytotoxic effect of the all the mycotoxin combinations increased in the following order: OTA+α-ZOL < ZEA+α-ZOL < OTA+ZEA < AFM_1+ZEA < AFM_1+α-ZOL < AFM_1+OTA < OTA+ZEA+α-ZOL < AFM_1+ZEA+OTA < AFM_1+ZEA+α-ZOL < AFM_1+OTA+α-ZOL < AFM_1+ZEA+OTA+α-ZOL. Isobologram analysis was further indicated that the existence of AFM1 brought additive effect at IC_{50} and even synergism at IC_{25}. The synergistic effect of the 4-toxin group is the highest of all mytotoxin combinations investigated (Combination Index = 0.17 ± 0.05 at IC_{25}). Other cell lines will be further studied in the future to obtain an appropriate health risk assessment of milk.

Key Words: aflatoxin M_1, cytotoxicity, interaction

T167 Clean-in-place cleaning validation at lower temperatures with alkaline chlorinated detergent Cool CIP. Gary Smith*, John Partridge, and Zey Ustunol, *Michigan State University, East Lansing, MI.*

Traditionally removal of organic soils in a clean-in-place (CIP) cleaning cycle by alkaline, chlorinated detergents is carried out at temperatures

ranging from 50 to 65°C. The objective of this research was to determine the cleaning efficiency of a newly formulated CIP solution called Cool CIP, a chlorinated alkaline cleaner developed by DeLaval Cleaning Solutions designed to function at low temperatures. Cool CIP is formulated to function at temperatures ranging from 32 to 40°C. The study was conducted over a 1-year period at the Michigan State University Dairy Plant. CIP cleaning cycles of a raw milk tanker truck and receiving lines, raw milk lines, and raw milk storage tanks were chosen to be included in the study. Effectiveness of Cool CIP was evaluated on each cleaning cycle by analysis of solution temperature, chlorine level, pre-wash and post-wash turbidity, Adenosine Tri-Phosphate (ATP) bioluminescence counts, as well as visual confirmation of overall cleanliness. Samples were collected at the beginning and end of the chemical recirculation step of each CIP cycle. ATP swabs were taken inside the vessel of each cycle, before and after each complete cleaning cycle. The results showed that Cool CIP was able to clean effectively at temperatures as low as 30°C as careful visual inspection of all systems passed after each cleaning cycle. The results also showed that all ATP tests were passable post-cycle on tests at 30°C. The raw line tanker truck receiving lines (n = 14) had 1011.6 ± 943.4 ATP units pre-cleaning, and 0 ± 0 ATP units post-CIP cleaning cycle. The raw milk lines (n = 7) had 10611.3 ± 25620.2 ATP units pre-cleaning, and 1.5 ± 4.8 ATP units post-CIP cleaning cycle. The raw milk tanks (n = 12) had 1381.1 ± 1116.2 ATP units pre-cleaning, and 0 ± 0 ATP units post-CIP cleaning cycle. This paper will present Cool CIP as an effective substitute for traditionally accepted high-temperature alkaline CIP cleaning regimens.

Key Words: clean-in-place (CIP)

T168 Metagenomic evidence of the prevalence and distribution patterns of antimicrobial resistant genes in dairy agroecosystems. Dipti Pitta*, Sanjay Kumar, Nagaraju Indugu, Zhengxia Dou, John Toth, Bonnie Vecchiarelli, and Bhima Bhukya, *Department of Clinical Studies, School of Veterinary Medicine, New Bolton Center, University of Pennsylvania, Kennett Square, PA.*

Antimicrobial resistance (AR) is a global problem with serious implications for public health. AR genes are prevalent on animal farms but little is known about their origin and distribution patterns in animal farm agroecosystems. In this study, a total of 20 resistomes (collections of AR genes), 5 each from animal feces, manure, near and far soil, on dairy farms were analyzed using shotgun approach. Antimicrobial resistance genes were detected on all 5 farms and only 2% was annotated to AR genes, but varied with sample type ($P < 0.05$). Despite variations in abundance, majority of AR genes (*acr*B, *bcr*A, *mac*B, *mdt*F, *pbp*1a, *tet*L, *tet*M, *tet*O, *tet*Q, *tet*S, *tet*W, *vanr*A, *vanr*B) that conferred resistance to multiple antibiotics, including macrolides, β-lactams, amphenicols, and tetracyclines, were common to all samples. Additionally, fecal and manure resistomes contained AR genes specific for quinolones, sulfonamides and aminoglycosides. Across all farms, manure was identified as a "Hot Spot" with the greatest abundance and diversity of AR genes. We compared AR genes from present data set with publically available data sets using hierarchal clustering. Multidrug resistant AR genes were prevalent across different animal species and humans, implicating an exchange of AR genes between unrelated microbial niches that may be potentiated by human activities. This study reports the prevalence of AR genes across different farm sectors and identifies potential pathways for horizontal transmission of AR genes (animal-manure-soil) in dairy agroecosystems.

Key Words: antimicrobial resistance, agroecosystems, antimicrobial resistance (AR) genes

T169 Antimicrobial resistance of *Escherichia coli* isolates from cheese made from unpasteurized milk in Brazil. Laryssa Freitas Ribeiro*[1,2], Mayhara Martins Cordeiro Barbosa[3], Fernanda de Rezende Pinto[4], Renato Pariz Maluta[5], Mônica Costa Oliveira[2], Viviane de Souza[6], Maria Izabel Merino de Medeiros[7], Lucimara Antonio Borges[2], Priscila Arrigucci Bernardes[1], Luiz Augusto do Amaral[2], and John Morris Fairbrother[1], *[1]Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada, [2]Universidade Estadual Paulista, Jaboticabal, São Paulo, Brazil, [3]Instituto Federal de Educação, Ciência e Tecnologia do Ceará, Quixadá, Ceará, Brazil, [4]Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil, [5]Universidade de Campinas, Campinas, São Paulo, Brazil, [6]Embrapa Caprinos e Ovinos, Sobral, Ceará, Brazil, [7]Instituto Tecnológico de Alimentos, Campinas, São Paulo, Brazil.*

The production of cheeses from unpasteurized milk is a public health problem, due to the use of raw milk and the associated poor hygienic conditions. Contamination may occur from several sources, involving several different pathogenic microorganisms, including *Escherichia coli*. The use of antimicrobials in animals has led to emergence of resistant microorganisms, contributing to the ineffectiveness of these products. The purpose of the current study was to investigate the presence of antimicrobial resistance in *E. coli* isolates in raw milk cheese in Brazil and to identify the potential risk to public health. A total of 83 cheeses from 3 different cities, Uberaba, Minas Gerais (30), Ribeirão Preto, São Paulo (22) and Aracaju, Sergipe (31) were cultured. From each cheese, 5 colonies were examined and a total of 169 *E. coli* isolates, 51, 25, and 93 from Uberaba, Ribeirão Preto, and Aracajú respectively were obtained. Ninety-five randomly selected isolates were tested for susceptibility to the 15 antimicrobials: amoxicillin + clavulanic acid (20 + 10 μg), ceftiofur (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), amikacin (30ug), ampicillin (10 μg), cefoxitin (30 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), streptomycin (10 μg), tetracycline (30 μg), chloramphenicol (30 μg), sulfisoxazole (0.25 mg) and trimethoprim + sulfamethoxazole (1.25 + 23.75 μg) used for testing generic *E. coli* in the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS), by the disk-diffusion (Kirby-Bauer) method. The greatest number of isolates with a resistance pattern (one antimicrobial class or more) was observed in Uberaba, most being resistant to 1–2 antimicrobial classes. Multidrug resistance (resistance to 3 or more classes of antimicrobial agents) was much more frequently observed in isolates from Uberaba and Ribeirão Preto (12 to 14%) than in those from Aracaju (2%). The highest prevalence of resistance in all regions was to tetracycline. Strikingly, prevalence of resistance to ampicillin and amoxicillin/clavulanic acid was much higher in Uberaba than in Ribeirão Preto and Aracajú. For example, 58.85% of Uberaba samples were amoxicillin/clavulanic acid resistant while Ribeirão Preto and Aracaju were 18.18% and 3.57% respectively. For ampicillin, the prevalence in samples were 61.54%, 27.27% and 14.29% to Uberaba, Ribeirão Preto and Aracaju repectively. All 95 isolates were susceptible to ceftriaxone, amikacin, gentamicin, and chloramphenicol. Resistance to ceftiofur was found in Ribeirão Preto, suggesting the therapeutic use this antimicrobial.For statistics analysis, the sample was considered resistant to the antimicrobial when at least one of the isolates demonstrated resistance and it was used exact chi-squared. So, with the test, *P*-value was 0.001571 and it showed that the city is not resistant independent, thus they do not have the same resistance. And it showed that Uberaba was different from Ribeirão Preto and Aracaju and Aracaju and Ribeirão did not differ ($P < 0.05$). The variation of resistance to antimicrobials between regions observed in the present study indicates

that the cheese made with unpasteurized milk in Brazil may contain *E. coli* that can be a risk for public health.

Key Words: public health, microbiology, antimicrobial resistance

T170 Ecoepidemiology of *Staphylococcus* spp. in small-scale goat milk dairy plants in northeastern Brazil. Candice de Leon[1], Celso Oliveira*[1], Iara Siqueira[2], Maria G. Carvalho[2], and Denis Spricigo[3], *[1]Federal University of Paraiba (UFPB), Brazil, Areia, PB, Brazil, [2]Federal University of Campina Grande, Patos, PB, Brazil, [3]LANAGRO, Porto Alegre, RS, Brazil.*

The microbiological quality of goat milk needs to be monitored to ensure the milk and milk products are safe for consumers. Our previous studies have demonstrated high contamination of goat milk by coagulase-positive and coagulase-negative *Staphylococcus* spp. that frequently harbor enterotoxin-producing genes. Staphylococci have been associated with food-poisoning outbreaks in humans and because staphylococci are commonly isolated from goat milk and are have an important effect on public health, these organisms are used as indicators of contamination in goat dairy plants. Therefore, this study aimed to evaluate the epidemiology of *Staphylococcus* spp. at different points of small-scale processing plants of goat milk in northeastern Brazil, the leading goat milk-producing region in the country. A longitudinal study was performed in 2 goat milk processing plants; samples of raw and pasteurized milk and swabs of the inner surface of equipment, walls, and handlers' hands were collected. Samples were collected in the morning before milk processing to detect residual contamination. *Staphylococcus* spp. isolates (n = 36) were subjected to genotyping by REP-PCR using the primer RW3A. Indistinguishable genotypic profiles were observed among *Staphylococcus* spp. collected from milk samples, equipment, and handlers' hands, indicating the presence of residual contamination during milk processing and the need to improve cleaning and disinfection procedures. The *Staphylococcus* spp. isolates from handlers' hand swabs had high genotypic relatedness with isolates from equipment and pasteurized milk, indicating that workers can play a role in the cross-contamination by *Staphylococcus* spp. in milk processing plants.

Key Words: food safety, genotyping, pathogen

T171 Effects of purple prairie clover (*Dalea purpurea* Vent.) on feed intake, nutrient digestibility and fecal shedding of *Escherichia coli* O157: H7 in lambs. Qianqian Huang1,2, Long Jin[1], Zhong Xu[1], Ruth Barbieri[1], Surya Acharya[1], Tianming Hu[2], Tim McAllister[1], Kim Stanford[3], and Yuxi Wang*[1], *[1]Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, [2]College of Animal Science and Technology, Northwest A&F University, China, [3]Alberta Agriculture and Rural Development, Lethbridge Agriculture Centre, Lethbridge, AB, Canada.*

This study was conducted to assess the effects of purple prairie clover (PPC, *Dalea purpurea* Vent.) on feed intake, nutrient digestibility, fecal shedding of *Escherichia coli* O157: H7 and blood metabolites of lambs. Three groups of lambs (6 per group)were individually fed green chop alfalfa (Alf), a mixture of Alf and PPC in a ratio of 40:60 (DM basis; Mix) and Mix supplemented with polyethylene glycol (Mix-P) for 45 d. Polyethylene glycol (PEG) was included to neutralize the biological activity of condensed tannins (CT) in PPC. Total-tract digestibility was determined using AIA as an indigestible marker. Blood samples were collected to measure serum metabolites and activity of antioxidant enzymes. All lambs were orally inoculated with a 5-strain mixture of *E. coli* O157:H7 after determination of diet digestibility and fecal samples were enumerated for *E. coli* O157:H7 after 1, 2, 3, 4, 7, 14, 21 and 28 d of inoculation. Feed intake was similar among lambs fed the 3 diets. The OM digestibility of Mix ($P < 0.05$) and CP digestibility of both Mix and Mix-P ($P < 0.01$) were lower than Alf. However, the 3 diets had similar NDF digestibility. After inoculation, lambs fed Mix shed less *E. coli* O157:H7 than lambs fed Alf between d 1 and d 7 ($P < 0.05$ to 0.006), and lambs fed Mix-P between d 1 and d 3 ($P < 0.05$ to 0.012). Lambs fed Alf shed more *E .coli* O157:H7 than lambs fed Mix ($P < 0.001$) or Mix-P ($P < 0.01$) over the 28-d post-challenge period. Lambs fed Mix had lower levels ($P < 0.05$) of serum creatinine and urea nitrogen than Alf, but both Mix and Mix-P had higher ($P < 0.05$) levels of serum P. Mix fed lambs had greater ($P < 0.05$) serum total antioxidant capacity but lower ($P < 0.05$) serum superoxide dismutase activity than lambs fed Alf. Incorporation of PPC in lamb diets at 60% of DM reduced fecal shedding in lambs challenged with *E. coli* O157:H7 as compared to Alf. The fact that shedding of *E. coli* O157:H7 was also reduced with Mix-P suggests that the anti- *E. coli* O157:H7 response of PPC is not solely due to CT.

Key Words: purple prairie clover, *E. coli* O157: H7, condensed tannins

T172 Reduction of biological hazards in animal feed mills via a decontamination protocol. Anne R. Huss*, Roger A. Cochrane, Aiswariya Deliephan, Charles R. Stark, and Cassandra K. Jones, *Kansas State University, Manhattan, KS.*

Animal feed and ingredients have been shown to be potential vectors of pathogenic bacteria in to the human food chain. Introduction of contaminated materials can lead to facility contamination, which can be easily spread to non-contaminated materials, including finished feeds. Upon detection of contamination, facility decontamination is necessary to prevent potential cross-contamination to feedstuffs. The purpose of this experiment was to evaluate a standardized protocol to decontaminate an animal feed manufacturing facility using *Enteroccoccus faecium* (ATCC 31282) as an indicator. A pelleted swine diet inoculated with *E. faecium* was manufactured at the Kansas State University Feed Safety Research Center. Environmental samples including swabs, RODAC plates and air samples were collected and consisted of: 1) baseline before inoculation, 2) after inoculated feed production, 3) after production physical clean using pressurized air, 4) after chemical clean with a quaternary ammonium-glutaraldehyde blend, 5) after chemical clean with sodium hypochlorite, 6) after facility heat-up to 60°C for 24 h, 7) 48 h, and 8) 72 h. Air samples collected on the exterior of the facility confirmed pathogen containment as *E. faecium* concentrations were equal to or lower than baseline levels at each sample location. Decontamination step and its associated interactions were the only factors that affected *E. faecium* incidence ($P < 0.0001$ vs. $P > 0.22$). After production of the inoculated diet, 85.7% of environmental samples were positive for *E. faecium*. Interestingly, physical cleaning had no effect on contamination ($P = 0.32$). Chemical cleaning with a quaternary ammonium-glutaraldehyde blend and sodium hypochlorite each reduced ($P < 0.0001$) *E. faecium* contamination to 28.6% and 2.4% of tested surfaces. All samples were negative after 48 h of heating. In summary, both wet chemical cleaning and facility heating resulted in substantial *E. faecium* decontamination, but not physical cleaning. In addition, this experiment confirmed both successful containment and decontamination of biological pathogens in the tested pilot-scale feed mill.

Key Words: feed safety, pathogen, decontamination

T173 Evaluation of select bacterial populations in poultry excreta and potential treatments for their disinfection. C. Arzola*[1], J. Corrales[1], R. Anderson[2], M. Hume[2], O. Ruiz[1], A. Corral[1], C. Rodriguez-Muela[1], Y. Castillo[3], J. L. Guevara[1], and R. Lechuga[1], *[1]Universidad Autonoma de Chihuahua, Chihuahua, Chihuahua, Mexico, [2]ARS,USDA, SPARC, College Station TX, [3]Universidad Autonoma de Ciudad Juarez, Casas Grandes, Chihuahua, Mexico.*

Because poultry litter has been used as feed supplement for cattle, there is interest in learning more about any unwanted bacteria present in poultry excreta. The objective of this study was to test for the presence of select bacterial populations in UACH poultry excreta for total aerobes, *Salmonella* spp., *E. coli* and coliforms, and *Campylobacter* spp. Additionally, sodium chlorate and nitroethane were evaluated as bactericidal agents to reduce bacterial concentrations. Approximately 45 kg of poultry excreta were mixed with 16 L of water and distributed equally to 9 buckets. Treatments were sodium chlorate (10 mM concentration), nitroethane (12 mM concentration) and a control. Excreta temperature and pH were measured. Samples were collected at 0, 6 and 24 h and processed for bacteriological enumeration. Enumeration of total aerobes, *Salmonella* spp., *E. coli* and coliforms, and *Campylobacter* spp. were performed with 3M Petrifilm Total Aerobe Count Plates, brilliant green agar supplemented with novobiocin, 3M Petrifilm E.coli/Coliform Count Plates and Campy-Cefex Agar, respectively. Data were analyzed by means of an ANOVA design, with time and treatments as main effects and their interactions. There was found a main effect of time ($P < 0.0001$) but not treatment or an interaction ($P > 0.05$) on litter temperature and pH. A main effect of time ($P < 0.0001$) was observed but treatment and the time x treatment interaction ($P > 0.05$) for litter temperature and pH was not significant. A main effect of time ($P < 0.01$) but not treatment or the interaction ($P > 0.10$) on total aerobes or on *Salmonella* spp. was observed. A treatment x time interaction ($P = 0.05$) on *E. coli* and total coliforms was observed. *Campylobacter* spp. was present but no effects of treatment or time ($P < 0.15$) on incidence of culture-positive samples was observed. *Campylobacte*r concentrations were not sufficient across all samples to perform quantitative analysis. In conclusion, modest effects of chlorate and nitroethane treatment were observed on *E. coli* and total coliforms but not on total aerobes or *Salmonella.*

Key Words: poultry excreta, *Salmonella* and *E. coli*, chlorate nitroethane

T174 *Trypanorhyncha* cestodes in *Brachyplatystoma rousseauxii*, *Cynoscion leiarchus*, *Cichla* spp., and *Colossoma macropomum*, captured in coast of Amazon/Brazil. Raquel L. Salgado*[1] and Josemir S. Gonçalves[2], *[1]Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil, [2]Universidade Federal Rural do Semi Árido, Mossoró, Rio Grande do Norte, Brazil.*

Fish consumption in the Amazon region is one of the world's largest; however, many species of fish commonly show high parasitism rates in muscles, making them unfit for consumption. In this context, the aim of this study was to evaluate the diversity of the parasitic fauna of 4 different species of fish captured in the northern coast of Amazon/Brazil. During the months from January to December 2009, 60 specimens of each species (*Brachyplatystoma rousseauxii*, *Cynoscion leiarchus*, *Cichla* spp., and *Colossoma macropomum*) were weighed, measured, and identified according their anatomic traits. They were filleted and with the aid of a candling table, their muscles were evaluated for the presence of parasites. The *Trypanorhyncha* larvae were released from their blastocysts, placed in Petri dishes containing distilled water for 24 h in the refrigerator. Subsequently, they were fixed in A.F.A. (95 parts of 70% alcohol, 5 parts of formalin, 2 parts of acetic acid) for 24 h. The cestodes identification was based in their morphologic traits. The amount of parasites in each specimen and overall were used to calculate the prevalence (P), infection intensity (I), mean infection intensity (MI) and mean abundance (MA). Of the 60 specimens of *B. rousseauxii* analyzed, 2 were infested by cetodes parasites, one by *Poecilancistrium caryophyllum*, P (1.60%), I (1), MI (1.00), MA (0.02) and one by *Pterobothrium heteracanthum* P (1.60%), I (1), MI (1.00), MA (0.02). Three specimens of *Cynoscion leiarchus* exhibited multiple parasitism by *Anisakis* spp. and *Poecilancistrium caryophyllum* P (7.50%), I (1–2), MI (1.50), MA (0.10). Six specimens of *Cichla* spp. were infested by *Clinostomum complanatum*, P (10.00%), I (2–3), MI (2.50), MA (0.92) and 2 are also infested by *Contracecum* spp. P (17.00%), I (1–3), MI (1.70), MA (0.27). No specimen of *Colossoma macropomum* was infested by any parasites. Despite they do not have zoonotic potential, the high prevalence of *Trypanorhyncha* represents a serious risk to human health, through the possibility of causing allergic reactions in humans.

Key Words: fish, human health, parasite

T175 Helminth with zoonotic potential in fish of Amazon/Brazil. Raquel L. Salgado*[1] and Josemir S. Gonçalves[2], *[1]Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil, [2]Universidade Federal Rural do Semi Árido, Mossoró, Rio Grande do Norte, Brazil.*

Among the parasites with zoonotic potential, nematodes from the family Anisakidae are most involved in cases of infection due to the consumption of fish. The aim of this study was to evaluate the presence of parasites with zoonotic potential in *Brachyplatystoma rousseauxii*, *Cynoscion leiarchus*, *Cichla* spp., and *Colossoma macropomum* captured in the northern coast of Amazon/Brazil. During the months from January to December 2009, 60 specimens of each specie were weighed, measured, and identified according their anatomic traits. They were filleted and with the aid of a candling table, their muscles were evaluated for the presence of parasites. Nematode larvae were placed in Petri dishes containing distilled water and subsequently fixed in A.F.A. (95 parts of 70% alcohol, 5 parts of formalin, 2 parts of acetic acid) for 24 h. The nematodes identification was based in their morphologic traits. The amount of parasites in each specimen and overall were used to calculate the prevalence (P), infection intensity (I), mean infection intensity (MI) and mean abundance (MA). Nematodes of the genus *Anisakis* spp. were observed parasitizing 15 specimens of *Cynoscion leiarchus* P (37.50%), I (3–42), MI (18.06), MA (6.77). Three specimens exhibited multiple parasitism by *Anisakis* spp. and *Poecilancistrium caryophyllum* P (7.50%), I (1–2), MI (1.30), MA (0.10). Twelve specimens of *Cichla* spp. were infested, of which, 10 by nematodes of the genus *Contracecum* spp. P (17.00%), I (2–12), MI (2.50), MA (0.92) and 2 by *Contracecum* spp. and by *Clinostomum complanatum* P (17.00%), I (1–3), MI (1.70), MA (0.27). Nine specimens of *B. rousseauxii* were infested by *Anisakis* spp. P (15.00%), I (4–32), MI (12.00), MA (1.80). No parasite species were found in the 60 specimens of *C. macropomum* analyzed. The parasites found may be responsible for allergic and gastric reactions and may even lead to death in humans. The high prevalence of parasites with zoonotic potential combined with the small number of studies on the parasite fauna of the Amazon fish, represents a serious risk to human health, and reinforce the need for a larger number of studies in this area.

Key Words: Anisakidae, human health, parasite