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SYMPOSIA AND ORAL SESSIONS

Animal Health I

21 Application of a novel biochip for rapid detection of mastitis-causing pathogens in bulk tank milk in Taiwan. K. H. Lee*[1], Y. M. Shy[1], Y. T. Lin[2], L. Y. Liu[2], S. J. Lee[1], C. L. Chang[1], M. C. Wu[1], and C. H. Chi[3], [1]*Hsinchu Branch, COA-LRI, Hsinchu, Taiwan, R.O.C.*, [2]*DR. Chip Biotechnology Inc., Chu-Nan, Taiwan, R.O.C.*, [3]*University of Taiwan, Taipei, Taiwan, R.O.C.*

In order to efficiently prevent and treat bovine mastitis and minimize its impact on dairy industry in Taiwan, a sensitive, rapid, and specific test is required for identifying the mastitis-causing pathogens. In the present study, a biochip was developed in collaboration with DR. Chip Biotechnology Inc. in Taiwan. The biochip is capable of detecting 6 common species of mastitis-causing pathogens within 6 hours, including Streptococcus bovis, Streptococcus uberis, Streptococcus uberis, Streptococcus dysgalactiae, Escherichia coli and Staphylococcus aureus. The technique is based on DNA amplification of genes specific to the target pathogens and consists of 4 basic steps: DNA extraction of bacteria, PCR reaction, DNA hybridization, colorimetric reaction. The sensitivity and accuracy of this Biochip, in comparison with conventional culture method, were verified by analyzing serial diluted fresh whole milk that were separately inoculated with the 6 species of bacteria that mentioned previously. The Biochip had a lower detecting limit and showed results that have a similar trend to those from the conventional method. Thereafter, the Biochip was used for detecting bacteria in bulk tank milk samples from 207 DHI-participating dairy farms in 2004. The results show that only 6 (3%) farms were negative on all 6 species of pathogens monitored. Streptococcus bovis, detected in samples from 161 (79.7%) farms, was the most prevalent species, followed by Streptococcus uberis (n=123, 60.9%), Escherichia coli (n=92, 45.5%), Streptococcus dysgalactiae (n=62, 30.7%), Streptococcus agalactiae (n=55, 27.2%), and Staphylococcus aureus (n=19, 9.4%). Results from this study reveal that the biochip is a feasible tool for a rapid diagnose of mastitis-causing pathogens in milk, which might prevent and cure mastitis more effectively.

Key Words: Mastitis, Pathogen, Biochip

22 Relationship of intramammary infection prevalence with somatic cell score in commercial herds. R. L. Bamber*[1], G. E. Shook[1], G. J. Bennett[2], Y. H. Schukken[2], and P. L. Ruegg[1], [1]*University of Wiscosin, Madison*, [2]*Cornell University, Ithaca, NY*.

Our objective was to derive predictions of intramammary infection (IMI) prevalence based on somatic cell score (SCS), cow, and herd factors. IMI cultures on composite samples were performed by New York Quality Milk Production Services during 1992 to 2004. Cow records containing DHI SCS in IMI sampling month with 0, 1, or 2 pathogens were retained. Herds were required to test at least 60% of cows and ≥ 30 cows tested. Final data set consisted of 79,308 cows from 1124 herds. Pathogens were classified as contagious or environmental. Models were derived using the glimmix macro in SAS with a logistic link function and employing backward elimination with $P < .01$. Three models were investigated with binary dependent variables: presence/absence of contagious IMI (CONT), environmental IMI (ENV), and all IMI (ALL). Independent variables included SCS, quadratic SCS (SCS^2), parity, days in milk (DIM), milk/day, J5 vaccine use, survey type, production system, season, year, herd (fitted as a random effect), and significant interactions of SCS with other terms. For all models SCS, SCS^2, parity, DIM, and production system were significant. In each model additional terms were: CONT: survey type; ENV and ALL: year, parity*SCS, and parity*SCS^2; ENV: milk/day and survey type; ALL: production system*SCS. Prevalence of IMI varied by herd and cow factors and increased at a decreasing rate as SCS increased. The rate of increase with SCS was uniform for CONT, but differed among production systems (ALL) and parities (ENV and ALL).

Table 1. Regression coefficients for IMI on SCS, herd and residual variances, and predicted prevalence (p) of IMI.

Model	a	b_1	b_2	Herd var.	Residual var.	SCS=2	SCS=4	SCS=6
CONT	-3.084	0.600	-0.042	1.711	0.839	0.144	0.205	0.270
ENV	-2.092	0.483	-0.027	0.403	0.966	0.225	0.356	0.459
ALL	-1.623	0.550	-0.025	0.601	0.972	0.349	0.544	0.685

Prevalence is shown for three levels of SCS for parity 2, DIM 60-120, years 2002–2004, voluntary survey, herds ≤ 240 cows in a freestall/parlor system, and 30 kg milk/day $(\ln(p/(1-p)) = a + b_1 SCS + b_2 SCS^2)$.

Key Words: Intramammary infection, Somatic cell score

23 An evaluation of DeLaval DCC for determining udder health status in dairy cattle. K. Leslie*, J. Yeung, R. Dingwell, A. Bashiri, N. Perkins, and E. Vernooy, *University of Guelph, Guelph, Ontario, Canada*.

The DeLaval Direct Cell Counter (DCC) is a rapid, portable test for the quantification of SCC in milk. This study was designed to compare the DeLaval DCC to traditional in-lab methods, and to determine its usefulness in early postpartum cows. Composite samples were obtained from 120 lactating cows. Samples were analyzed using three methods for SCC determination: Fossomatic 5000, Bentley SomaCount 300 and the DeLaval DCC. A subset of these samples was tested using the

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DeLaval DCC both on-farm and in the laboratory. In addition, 42 cows on two farms were enrolled on the first scheduled visit to the farm after their calving (1 – 4 DIM). Three additional samplings were performed on each cow over the next two weeks. At the time of initial and subsequent sampling, a CMT test was performed, and a milk sample was aseptically collected from each quarter. The samples were transported to the University Lab for standard milk bacteriological culture, Bentley SomaCount SCC determination and the DeLaval DCC test. Results were recorded and analyzed using Microsoft Excel. Log SCC data was plotted for the DeLaval DCC instrument versus the Bentley SomaCount 300 and the Fossomatic 5000 electronic SCC instruments. There was extremely high correlation between DeLaval DCC and the standard laboratory SCC instruments, with a coefficient of correlation of 0.92 in both cases. A subset of 36 cow composite samples was evaluated with the DeLaval DCC instrument both on-farm and in the lab. There was reasonably good correlation ($R^2 = 0.87$), indicating that on-farm use of the DeLaval DCC instrument could work well. In addition, the mean and standard deviation of the DeLaval DCC and laboratory SCC data were compared to the CMT results from postpartum cows. Both the DeLaval DCC and Bentley SCC instruments were well correlated with the CMT results. The decline of log SCC as days postpartum increased is precipitous, and equivalent using both instruments. These data suggest that the DeLaval DCC instrument could be used as a monitoring tool for udder health in the postpartum period, and for lactating cows in general.

Key Words: SCC, CMT, Rapid test

24 Effect of winter housing on cow dirt score, somatic cell score and mastitis incidence in dairy cows. K. O'Driscoll*[1,2], L. Boyle[1], P. French[1], B. Meaney[1], and A. Hanlon[2], [1]*Dairy Production Research Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland*, [2]*School of Agriculture, Food Science and Veterinary Medicine, NUI Dublin, Belfield, Dublin 4, Ireland*.

The aim of this study was to evaluate three overwinter housing options for spring calving dairy cows; indoor cubicles [IC], uncovered woodchip pad [UP] ($12m^2$/cow) and sheltered woodchip pad [SP] ($6m^2$/cow), with regard to cow dirt score, incidence of mastitis, and somatic cell score (SCS). Animals (n=147) were randomly assigned to the housing treatments from 6 Dec until calving (median = 14 Feb), and scored for dirtiness (1 to 5 in half score increments) prior to housing, then every 14 days until calving. Cows were quarter milk sampled (QMS) for microbiology and somatic cell count (SCC) prior to housing, at drying off and approx. 3 weeks post partum. QMS were also collected 2.2 ± 1.98 days post calving and assayed for California Mastitis Test (CMT) and microbiology. Clinical mastitis (CM) was diagnosed when macroscopic changes in the milk or udder were observed. Subclinical mastitis (SCM) was diagnosed in the event of SCC > 500,000 or CMT > 2, without macroscopic changes. SCC was recorded at 2 week intervals throughout the following lactation. A log transformation of SCC to SCS was used to normalize the data distribution. Average SCS was calculated for three stages of lactation: 6 to 60 days in milk (DIM) (early), 61 to 220 DIM (mid), and 221 to 305 DIM (late). Dirt scores and SCS were analyzed using the Mixed Procedure of SAS. Differences in the incidence of CM, SCM, and the incidence of pathogens detected in QMS were analyzed using Fishers exact probability test. Housing had an effect on animal dirtiness, with SP cows dirtiest, then UP, and IC cleanest ($P < 0.001$). There were no cases of CM prior to housing, at dry off or during the housing period, and no difference in incidence of SCM. Three weeks post calving combined incidence of CM and SCM was higher in SP than UP or IC

($P < 0.05$), and a higher number of cows from SP had an infectious agent isolated ($P < 0.05$). However there was no effect of treatment on SCS over the following lactation.

Key Words: Winter housing, Somatic cell count, Cow dirtiness

25 Intramammary infection affects incidence of clinical infection after infusion of *Streptococcus uberis*. K. M. Sanders[1], S. McDougall[2], L. R. McNaughton*[1], G. E. Stanley[1], D. L. Johnson[1], S. Harcourt[1], and R. J. Spelman[1], [1]*Livestock Improvement Corporation Ltd, Hamilton, Waikato, New Zealand*, [2]*Animal Health Centre, Morrinsville, Waikato, New Zealand*.

Mastitis has a major impact on farm management and profitability in dairy production systems; therefore it is valuable to understand factors affecting susceptibility to infection. *Streptococcus uberis* is the most common pathogen causing clinical mastitis in New Zealand. The objective of this study was to identify factors contributing to clinical mastitis risk in the week after infusion in 263 F2 Friesian-Jersey cows. Animals were the daughters of 6 sires and averaged 109 days in milk at the time of infusion. Factors examined for their contribution to risk included sire, days in milk at infusion, SCC on the morning of infusion, mastitis treatment history, and the presence of any major pathogens at the time of infusion. 7-10 wks prior to infusion, milk samples were collected from all glands for bacteriology assessment, and antibiotic treatments were administered to clear infections. Treated glands were not selected for infusion. One gland from each cow was infused with an average of 104 colony-forming units (sd=22) of *Streptococcus uberis*. Milk samples were collected prior to infusion and at each milking post-infusion for 13 milkings, or until diagnosis of clinical mastitis. Logistic regression and survival analysis were used to determine risk factors. Overall, the cumulative incidence of clinical diagnosis was 71.9% (189/263) and the mean interval to clinical diagnosis was 66.2 (sd=36.5) hours. Sire had a significant ($P < 0.05$) effect on the incidence of clinical mastitis. Daughters of one sire had a significantly lower incidence of clinical mastitis. Results also showed that cows with a somatic cell count at infusion of ≥100,000 cells/ml had a lower incidence of clinical mastitis (18.3% vs. 81.5%; $P < 0.0001$). Infection status at infusion also had an effect on the incidence of clinical mastitis. The presence of *Staphylococcus aureus* at infusion for cows that had no pre-infusion mastitis treatment(s) resulted in a lower incidence of clinical mastitis (29.0% vs. 82.8%; $P < 0.0001$). Further research will be conducted to identify the causative mechanism behind the protective effect of infection at infusion.

Key Words: Mastitis, Lactation, Staphylococcus aureus

26 Antimicrobial susceptibility patterns and trends in resistance development in bacteria isolated from milk, 2000-2004. P. J. Rajala-Schultz*[1] and B. C. Love[2], [1]*The Ohio State University, Columbus*, [2]*Penn State University, University Park*.

Antimicrobial resistance has been a growing concern worldwide. The objective of this study was to evaluate whether susceptibility of mastitis pathogens has changed in recent years. Milk from dairy cattle, submitted to the Pennsylvania State University Animal Diagnostic Laboratory (PSU ADL) from 2000 through 2004, was cultured for bacterial pathogens. Minimum inhibitory concentrations (MIC) were determined for 2554 isolates, using a commercially-available broth microdilution method (Sensititre, Trek Diagnostics, Westlake, OH) and interpretations of sensitive, intermediate or resistant were assigned according to guidelines published by the Clinical and Laboratory

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Standards Institute (CLSI). Isolates analyzed in this study included Staphylococcus aureus (N=242), coagulase negative staphylococci (N=367), Streptococcus uberis (N=393), Streptococcus dysgalactiae (N=304), Escherichia coli (N=299) and Klebsiella pneumoniae (N=261). Preliminary analyses indicate that a significant increase in resistance to beta-lactam-containing antimicrobials could be detected in streptococci, although not in staphylococci. Most of the antimicrobials included in the commercial mastitis panel have efficacy only against gram-positive organisms. Neither E. coli nor K. pneumoniae exhibited increasing resistance against ceftiofur or cephalothin over the years studied. It is generally accepted that antibiotics are beneficial in treatment of mastitis caused by gram-positive organisms. However, due to cost and the length of time required to identify the causative organism at a reference laboratory, and the potential of decreased efficacy if there is a delay in initiation of treatment, antibiotic treatment is frequently initiated without the knowledge about the causal agents. The increase in resistance to beta-lactam antibiotics seen in this study requires further study.

Key Words: Antimicrobial, Resistance, Mastitis

27 Neutrophil extracellular trap formation: An important neutrophil killing mechanism that is not inhibited by milk. J. Lippolis*, T. Reinhardt, J. Goff, and R. Horst, *National Animal Disease Center /ARS/USDA*, *Ames, IA*.

Neutrophils play a crucial role in protecting the mammary gland against bacterial infections. However, neutrophils incubated in milk have a decreased ability to kill bacteria in vitro and a decreased capacity to generate reactive oxygen species upon stimulation. Recently, a new neutrophil bacterial killing mechanism was described. When stimulated, human neutrophils release nuclear and granule material, and this extracellular material form webs that act as nets to trap and kill bacteria. We now show that bovine neutrophils form extracellular nets when stimulated by staining of extracellular DNA with Sytox-Orange and quantitated with a fluorescence plate reader. Furthermore, neutrophil extracellular traps can be formed even when neutrophils have been incubated for up to 6 hours (see Table). Extracellular nets formation in milk is not different from net formation in media. Stimulation of neutrophils with bacteria common to mammary gland infections (S. aureus S. marcescens and two strains of E. coli) leads to the formation of the neutrophil extracellular nets in milk or culture media. In fact some bacteria (E. faecalis, S. dysgalactiae and one E. coli) were able to stimulate enhanced formation of extracellular traps in milk compared to culture media (student two-tailed T-test, $P < 0.05$). In contrast to other neutrophil functions that are inhibited by milk such as phagocytosis and oxidative burst, neutrophil extracellular traps may be an important innate immune mechanism in mammary infections because this ability remains intact in the milk environment.

Table 1. Effect on NET Formation of Pre-incubation of Neutrophils in Media or Milk

Environment	Time Prior to Stimulation (Hours)	Stimulation Index (Stimulated/Un-stimulated)
Milk	0	2.0
Media	0	2.4
Milk	1	2.4
Media	1	3.0
Milk	2	3.5
Media	2	3.5
Milk	4	3.4
Media	4	3.4
Milk	6	2.1
Media	6	3.1

Neutrophils from 12 cows were incubated in milk of media for various times, then stimulated (PMA+Ionomycin) or not in 6 replicates. Extracellular DNA was determined by SytoxOrange staining. No significant difference in NET formation was seen between Milk and Media (Repeated measures ANOVA with Time using Tukey's adjustment for multiple comparisons).

Key Words: Neutrophil, Milk, Antibacterial

28 Hepatic ApoB100 and ApoE mRNA in periparturient dairy cows. U. Bernabucci*[1], B. Ronchi[1], L. Basiricò[1], D. Pirazzi[1], F. Rueca[2], N. Lacetera[1], E. Lepri[2], and A. Nardone[1], [1]*DiPA, Università della Tuscia*, *Viterbo, Italy*, [2]*Veterinary Medicine, Università di Perugia*, *Perugia, Italy*.

Previous research suggested that decreased mRNA for apolipopro-tein-B100 (ApoB100) in transition cows may be consistent with decreased synthesis and/or secretion of very low density lipoproteins from liver during the periparturient period. The aim of the present study was to evaluate changes of hepatic ApoB100 and apolipoprotein-E (ApoE) mRNA in periparturient cows, and to evaluate possible relationship between apolipoproteins gene expression and liver status. Sixteen multiparous Holstein cows were monitored during the transition period. From -32 to 38 d relative to calving, body condition score (BCS) was registered, and plasma indices of energy metabolism (glucose, BHBA and NEFA) were determined to evaluate metabolic status. Liver biopsies were performed on d -32, 3, and 38 relative to calving. Gene expression of ApoB100 and ApoE was determined on liver tissue by Ribonuclease Protection Assay method; triglycerides (TG) accumulation in the liver was determined by histochemical method. After calving, BCS and plasma glucose decreased ($P < 0.01$), and plasma NEFA and BHBA increased ($P < 0.01$). Compared with values of day -32, synthesis of the ApoB100 mRNA was lower ($P < 0.05$) on 3 and 38 d after calving, whereas that of ApoE mRNA was higher ($P < 0.05$) on d 3 after calving. At 3 d after calving, positive relationships between plasma NEFA (r=0.42, $P < 0.01$) or BHBA (r=0.49, $P < 0.01$) with liver TG accumulation and negative relationship (r =-0.29, $P < 0.05$) between plasma glucose and TG accumulation were detected. In addition, at 3 d after calving negative (r =-0.59, $P < 0.01$) correlation between liver TG and ApoB100 mRNA abundance was observed. No relationship between ApoE and liver TG accumulation on d 3 after calving was observed. The study indicates that the accumulation of liver TG, occurring in dairy cows after calving, is associated with down-regulation of ApoB100 expression.

Key Words: ApoB100 ApoE mRNA, Liver, Dairy cow

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29 Effect of isoflupredone acetate with or without long acting insulin on postparturient energy metabolism in lactating dairy cows. H. Seifi[1], S. LeBlanc[2], K. Leslie*[2], and T. Duffield[2], [1]*School of Veterinary Medicine, Ferdowsi University of Mashhad*, *Iran*, [2]*Ontario Veterinary College, University of Guelph*, *Canada*.

Glucocorticoids are commonly used to treat cows with clinical ketosis and fatty liver disease. This study investigated the effects of isoflupredone acetate (IA), alone or with long-acting insulin, on the energy metabolism of early postpartum dairy cows. A total of 1162 Holstein cows and first lactation heifers received, by double blind random assignment, one of three treatment regimens between the day of calving and 8 DIM. The treatments were: 20 mg IA IM plus 100 units of insulin SC; 20 mg IA IM plus sterile water SC; 10 ml sterile water IM plus 1 ml sterile water SC (controls). Serum samples obtained at the time of treatment, and at weeks 1 and 2 following treatment, were analyzed for β–hydroxybutyrate (BHBA), non–esterified fatty acids (NEFA), glucose, calcium, potassium, sodium and chloride. Data were analyzed using a repeated measures mixed model that accounted for the effects of parity, BCS, and the random effects of cow and farm. BHBA and NEFA concentrations at 1 week following treatment were significantly higher ($P < 0.02$) in both treatment groups compared to control cows. In addition, cows that received IA plus insulin had lower glucose ($P < 0.01$) concentrations at 1 week after treatment. Calcium concentrations were significantly lower ($P < 0.01$) for cows treated with IA plus insulin, and IA alone, in week 1. Sodium, potassium and chloride concentrations were not influenced by treatment. Among 190 cows that were ketotic before treatment, neither treatment improved resolution of subclinical ketosis (SCK; [3] 1400 µmol/l) relative to control animals. In fact, cows receiving both IA and insulin tended (OR=2.0, $P = 0.06$) to be more likely than controls to remain ketotic at 1 Week after treatment. Among 972 cows that were not ketotic at enrollment, cows that received IA plus insulin, or IA alone, were 1.7 and 1.6 times more likely, respectively, to develop SCK 1 week after treatment. The results of this study indicate that IA plus insulin, or IA alone, was not effective for either the prevention or alleviation of subclinical ketosis in postpartum dairy cows.

Key Words: Subclinical ketosis, Therapy, Prevention

30 Use of rectal temperature monitoring to identify post-partum metritis in dairy cattle. J. R. Wenz*, S. M. Scott, S. E. Dobberstein, and W. Wailes, *Colorado State University*, *Fort Collins*.

Rectal temperature monitoring (RTM) for the first 10 DIM has been advocated as a management tool to identify cows with post-partum disease, especially metritis. The objective of this study was to comparing the ability of this time consuming practice (RTM) with visual observation to identify cattle with metritis. Rectal temperature and milk production were recorded for the first 10 and 30 DIM respectively of 208 Holstein cows. Cows with temperatures >103.0°F (FEVER) were further examined for metritis (MET). MET diagnosed by dairy staff using visual observation (VO), calving ease, 5-25 DIM production (5-25dMLK) and first service conception rate (CR1) were also recorded. During their first 10 DIM, 40% of cows had FEVER. Of cows with FEVER, 41% had MET. More cows were diagnosed with MET by RTM (16%) than by VO (5.8%) (P=0.0014). More cows

with dystocia (49%) had FEVER vs. those without (35%) ($P = 0.047$). More cows had FEVER DIM 1-5 (22%) vs. DIM 6-10 (5.8%) ($P = 0.0001$) and only 2 of the 12 cows with FEVER during DIM 6-10 had MET. Cows with MET diagnosed by RTM made 304lbs less 5-25dMLK than those without MET ($P = 0.0476$), however, there was no difference in CR1 between the two groups. Percent cows with FEVER peaked on DIM3 (14%) and DIM4 (23%) for cows without and with dystocia, respectively. There was no difference in FEVER during DIM1-3 between cows with or without dystocia and many cows with FEVER during this time had MET. Twenty-nine % of cows had one consecutive day with FEVER and 23% of those cows had MET while 13% of cows had 2 or more consecutive days with FEVER and 70% of those cows had MET. These results suggest many cows with FEVER identified by RTM have MET and reduced milk production. Rectal temperature monitoring was more effective than visual observation for detection of metritis and may significantly improve the health and productivity of post-partum dairy cows.

Key Words: Fever, Metritis, Fresh cow

31 Rectal temperature measurement versus peripheral temperature sensing using radio-frequency implants in periparturient dairy cattle. E. D. Reid*, K. E. Karvetski, J. M. Velasco, R. L. Wallace, and G. E. Dahl, *University of Illinois*, *Urbana*.

The periparturient cow is challenged by changing metabolic processes driven by parturition and the ensuing lactation. These challenges leave the animal more susceptible to both metabolic and pathogenic insults. The ability to detect disease earlier may result in faster treatment times and be associated with higher treatment success. Injectable radio frequency implants (RFI) with the ability to remotely monitor temperature at the site of implantation are available. Temperatures recorded from these RFI exhibit a positive correlation with baseline rectal temperatures and a negative correlation to rectal temperature when cattle are challenged with lipopolysaccharide. We hypothesized that the RFIs, implanted under the scutiform cartilage of the ear of periparturient cows, would be positively correlated to baseline rectal temperature and would be negatively correlated to rectal temperature during a known health event. We also hypothesized that increased temperature sampling would allow for quicker identification of disease in these periparturient cows relative to traditional methods. Multiparous dairy cattle (n=40) were implanted with an RFI one wk prior to dry off. Rectal and RFI temperatures (RT and RFIT) were recorded every 12 h until –7 d before expected calving date when sampling frequency increased to every 6 hours until 14 d after calving. Ambient temperature (AMB) was also logged at 30 min intervals and was included in the statistical model. Mean RT (°C) varied throughout the day (39.7 ± 0.2, 39.3 ± 0.1, 38.7 ± 0.2, 39.9 ± 0.1) for 03:00, 09:00, 15:00 and 21:00 respectively. Mean RFIT also varied (37.7 ± 0.2, 37.5 ± 0.2, 37.6 ± 0.2, 38.1 ± 0.2) for the same time points. RFIT were positively correlated with RT (r=0.46, $P < 0.001$) and AMB was positively correlated with both RT and RFIT r=0.41 and 0.55, $P < 0.001$ respectively). A negative correlation between RFIT and RT during diagnosed health events (n=11 total; 4 metabolic, 7 reproductive) was not consistently observed therefore this approach has limited value in early disease detection.

Key Words: RFID, Temperature