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Poster presentation

P1 Cell Cycle Analysis of Cultured Skin Fibroblasts from the Leopard *(Panthera pardus)*

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Keywords: *cell cycle synchronization, leopard, skin fibroblast, somatic cell nuclear transfer*

Introduction and Objectives

The G0/G1 stage of the cell cycle is one of the most important factors determining the success of the development of cloned embryos (1-3). The cell cycle can be synchronized using serum starvation, contact inhibition and chemical inhibition (4-6). However, there is no study of the cell cycle synchronization of the leopard *(Panthera pardus)* which is one of the wild cats at risk of extinction. Our objective was to analyze the cell cycle of fibroblast cells of leopard cat

Materials and Methods

The leopard cells in passages 2nd to 3rd of culture were used in this study. Cells in each passage were treated by one of three methods as 1) serum starvation, culturing 60-70% confluency cells with Dulbeccoís modified Eagle's medium (DMEM) containing 0.5% fetal bovine serum (FBS) and observed every 24 h for 5 days, 2) cell confluency-contact inhibition, additional 5 days in culture after confluency had reached to 100% and 3) chemical inhibitor, adding roscovitine in different concentrations, (7.5, 15 and 30 μM, diluted with dimethylsulphoxide (DMSO), in the culture medium and treated for 24 h. DMSO toxicity was tested using 30 μM DMSO. Cycling cells were observed as the control. Cells from each treatment were disaggregated with 0.5 ml of 0.5% trypsin prior to fixation. Cells were analyzed using a flow cytometer.

Result and Discussion

Serum starvation for 4 days and induction of 100% confluency provided a higher percentage of leopaerd fibroblast cells at the G0/G1 phase (96 and 93.1%, respectively; Table 1). Adding chemical inhibitors such as roscovitine is able to arrest cells at the G0/G1 phase of the cell cycle. In the present study, 15 μM roscovitine gave a higher percentage of G0/G1 cells (89.95%; Table 2) compared to other concentrations (7.5 and 30 μm). This information will be useful for studying the donor cells cycle phase on the success of somatic cell nuclear transfer (SCNT) in wild cats.

Table 1 Mean percentages of leopard skin fibroblasts of 2 replications (passage 2nd and 3rd) in the various phases of the cell cycle after treatment with serum starvation and confluency-contact inhibition.

Cell cycle phase	Cycling	Culture treatment					
		Contact inhibition	Serum starvation (d)				
			1	2	3	4	5
G0/G1	88.6	93.1	95.15	94.25	94.25	96	95.9
S	4.7	1.5	0.45	0.6	0.75	0.4	0.3
G2/M	6.7	5	3.75	3.95	4.1	2.6	3.1
Apoptosis	0.15	0.5	0.8	1.3	0.9	0.9	0.6

**Value presented in percentage*

Table 2 Mean percentages of leopard skin fibroblasts of 2 replications (passage 2nd and 3rd) in the various phases of the cell cycle after treatment with roscovitine.

Cell cycle phase	Cycling	Culture treatment			
		DMSO	Roscovitine (μM)		
		30 μM	7.5	15	30
G0/G1	88.6	86.85	80.9	89.95	86.2
S	4.7	2.6	3.4	2.7	2.4
G2/M	6.7	10.1	15.6	6.6	9.4
Apoptosis	0.15	0.2	0.15	0.6	1.8

**Value presented in percentage*

References

1. Boquest, A.C. et al. 1999. Biol Reprod 60: 1013-1019.
2. Gómez, M.C. et al. 2003. Biol Reprod 69: 1032-1041.
3. Hashem, M.A. et al. 2006. Cell Biol. Intl. 30: 698-703.
4. Wilmut, I. et al. 1997. Nature 385: 810-813.

P2 Microsatellite-based Parentage Control in Swine

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Keywords: *microsatellites, parentage control, swine*

Introduction and Objectives

Swine are economically important to Thailand. They are highly fecund and produce 14-23 offspring per year. Improvement in breeds and breeding in swine very much depends upon information and the correct database on the pedigree of and relationships among sires, dam and offspring. Microsatellite loci which are simple nucleotide repeats, are highly polymorphic and can be used as DNA markers in parentage identification. This study aims to evaluate the efficiency of the microsatellite markers of swine raised in Thailand, in order to develop appropriate techniques in parentage control.

Materials and Methods

DNA from 80 pigs raised in Chainart and Rachaburi were isolated and microsatellite loci were amplified by PCR using 10 primers specific for microsatellites of 200-600 bp (1). PCR products were size-determined using polyacrylamide gel electrophoresis (PAGE). Analyses of allelic polymorphism for each marker were performed using observed/expected heterozygosity (H_{obs}/H_{exp}), polymorphic information content (PIC) and combined exclusion probability (CEP), in order to evaluate the efficiency in parentage testing.

Table 1 Characterizations of 10 microsatellite primers chosen in the analysis

Locus name	Primer sequences (5'-3')	Base repeats	Size (bp)
SJ859	F: 5'TCA AGA GAA AAG GAC AAA ATC 3' R: 5'AT GAA GAG GTG GAG ACT GTG 3'	$(TTTG)_3$	334
SJ923	F: 5'CCA AGA AAT AGC AAC AAC AA 3' R: 5'AGA TGA TTT GGT TTG GTC TTA 3'	$(CAA)_9$	197
SJ924	F: 5'GAT TTG TTT CCG CTG AGC CA 3' R: 5'TGG GCT CAC AGG CAC AGT ATC 3'	$(AAC)A(AAC)_6$	230
SJ925	F: 5' CAC AAA GGA GGA GGC TGG AAT 3' R: 5'TT GCT GTG GTC TGG CGT AGG 3'	$(TTG)_9$	383
SJ926	F: 5'CTA CCA CTG AGC CAC AAG AG 3' R: 5'TGG TGT AGA TTT CAG ATG CTG 3'	$(TTA)_9$	241
SJ927	F: 5'CTC AGT GTG GCA TTC AGG TC 3' R: 5'TGA CCT ACA CCA CAG CTC ATG 3'	$(TTG)_6$	272
SJ929	F: 5' ATG ACC CAG GAA CAA GGA TAG 3' R: 5'TC AAA GAA ATG GGG AAA CAG 3'	$(TTTG)TT(TTTG)_4$	327
S0719	F: 5'TCT CCA AGT CCA GGA ACT TGC 3' R: 5'TCG CCA TAC TCT TCT AAT GGC 3'	$(GAAA)_{11}$	600
S0766	F: 5'GTG TAG ATA TGT GTC TGT ACA 3' R: 5'AGA CCT CCT ATT AGA GGT GGA 3'	$(GAAA)_4(CA)_6$ TACACG $(CA)_{16}$	620
NLRIP0001	F: 5'GAT CTC AGC TTC AAT ACC TCC 3' R: 5'GAT CCT GTA TTG CTG TGG CTG 3'	$(TTTC)_{24}$	344

Results and Discussion

All microsatellite markers (except SJ925) used in this study could be amplified by PCR but there were only 3 loci (S0719, S0766 and NLRIP0001) which showed polymorphism in which the number of alleles for each locus ranged from 4-6. The Hobs/Hexp, PIC and CEP values of these 3 markers were 0.3250-1.000/0.6345-0.7729, 0.5665-0.6926 and 0.8412 respectively.

500
400

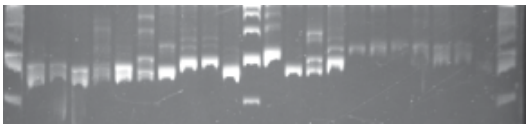

Figure 1 S0719 (no. of alleles = 5)

500
400

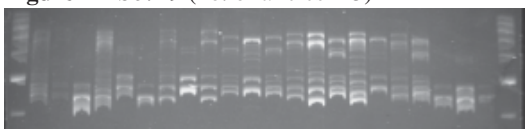

Figure 2 S0766 (no. of alleles = 4)

500
400
300

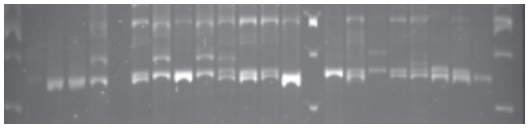

Figure 3 NLRIP0001 (no. of alleles = 6)

Of the 10 microsatellite loci used in this study, 9 were amplified and only 3 markers showed allelic polymorphism (Figs. 1-3). The number of alleles for each locus ranged from 4-6, indicating that their degree of polymorphism was moderate (2). Analyses of Hobs, Hexp and PIC values indicated that these 3 markers could be used in parentage testing with S0719 appearing to be the most efficient marker. However, when the CEP value (84.12%) of all 3 loci is considered, it is suggested that these markers were of low to medium efficiency (2, 3).

References

1. NCBI. 2008. http://www.ncbi.nlm.nih.gov/
2. Putnova, L. et al. 2003. Czech. J. Anim. Sci. 48: 307-314.
3. Rohrer, G.A. et al. 2007. Anim. Genet. 38: 253-258.

P3 Viability and Mitochondrial Activity of Testicular Sperm in Domestic Cats after Cold Storage

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Keywords: *cat, cold storage, mitochondria, testicular sperm, viability*

Introduction and Objectives

Testicular sperm are usually non-motile and the selection of viable sperm is thus difficult, especially when cold storage of testicular tissue at 4°C is required. The aim of this study was to examine the effect of cold storage on the viability and mitochondrial activity of testicular sperm.

Materials and Methods

In experiment I, 98 testes were stored at 4°C for 1 to 10 days in Dulbeccoís Phosphate Buffered Saline (DPBS) supplemented with antibiotics. Viability was assessed by Ethidium homodimer-1 (a membrane-impermeable DNA staining). Hoechst 33342 was used for counterstaining.

In experiment II, testicular sperm from 24 testes was extracted at 0, 2, 4 and 6 days after cold storage. Viability and mitochondrial activity were examined using DNA staining (DAPI) and MitoTracker Red CM-H2XRos (MTred), respectively.

Results and Discussion

The mean percentage of sperm viability extracted within 1 hr was 76.8±3.6% and did not significantly differ from sperm recovered within 6 hrs (79.4±3.7%) and 1-day after cold storage (67.6±2.2%). The viability gradually decreased to 37.3% during 10 days of cold storage. The viable sperm could found in either positive or negative staining with MTred, however 99% of the spermatozoa in positive MTred staining were classified as viable sperm.

Feline testes could be preserved at 4°C while the sperm viability remained in an acceptable range. The MTred binds specifically to active mitochondria and can be used to select viable sperm. Fertilizability of this MTred positive sperm will be elucidated.

References

1. Hallap, T. et al. 2005. Theriogenology. 63: 2311-2322.
2. Kumi-Diaka, J. and Badtram, G. 1994. Theriogenology. 41: 1355-1366.

P4 *In vitro* and *In vivo* Development of Flat-headed cat *(Prionailurus planiceps)* Cloned Embryos

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Keywords: *cell lines, embryo development, flat-headed cat, nuclear transfer*

Introduction and Objectives

The flat-headed cat (FC) is one of the critically endangered wild cats of Southeast Asia. Inter-generic nuclear transfer (ig-NT) using domestic cat (DC) oocytes to produce offspring is still promising in conservation strategies. However, many unknown factors including the ability of individual cell lines and the gender of donor cells on NT success have not yet been clarified. The objectives of the study were to investigate 1) the effect of the individual cell line and the gender of donor cells on FC cloned embryo production and 2) the pregnancy establishment of recipients receiving cloned FC embryos with or without DC IVF embryo co-transfer. The DC IVF embryos were used as a control.

Materials and Methods

Study I Three cell lines of FC fibroblasts (passage 3-5) collected from 2 females (L1 and L2; biopsied from muscle and skin, respectively) and a male (L3; biopsied from skin) were used as donor cells for nuclear transfer. The donor cells were fused with enucleated DC oocytes. The fused couplets were activated by the induction of 3 pulses of 1.2 kV/cm for 50 μs and subsequently incubated in an activation medium. Reconstructed embryos were cultured in SOFaa medium supplemented with 5% FBS at 38.5°C in air, and monitored for 7 days.

Study II Estrus and ovulation were induced in 15 DC recipients using 100-150 IU of PMSG and 100 IU of hCG (s/c). Recipients were divided into 3 groups; 1) a cloned group (n=5) receiving FC cloned embryos (mean 41.4±13), 2) a co-transferred group (n=4) receiving FC cloned and DC IVF embryos (mean 55±15; 43.3±15 of FC cloned and 10.8±1.5 of DC IVF embryos), and 3) an IVF/control group (n=6) receiving only DC IVF embryos (mean 25±9). Control DC IVF embryos were produced by co-incubation of DC oocytes with fresh DC semen for 18 h. Day-1 embryos were transferred into the oviducts of the recipients.

Results and Discussion

Study I Greater cleavage numbers ($p<0.05$) were observed when L1 was used as donor cells rather than L2 and L3. Developmental success to the morula stage of the embryo reconstructed from L1 was greater ($p<0.05$) than that for L3 but not L2 ($p>0.05$). However, there was no difference in blastocyst formation success among cell lines. The development of the embryos derived from female and male donor cells at subsequent stages was not different.

Study II Pregnancy evaluation using ultrasonography at day 30 post-transfer demonstrated that pregnancy was not observed in any recipients in the cloned group. One recipient from the co-transferred group became pregnant and delivered DC IVF stillbirths (n=2) and live kittens (n=6). All recipients in IVF group became pregnant and 3 recipients delivered 5 DC kittens.

Variations of fusion efficiency and embryo developmental success of FC couplets were observed between the cell lines. These results indicate that 1) the individual cell line but not the gender of the donor cells influences the development of ig-NT FC embryos and 2) by with or without co-transfer of FC cloned and DC IVF embryos, FC cloned offspring were not produced in the study.

Acknowledgements

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Reference

1. Thongphakdee, A. et al. 2006. Reprod. Dom. Anim. 41: 219-226.

P5 Induction of Porcine Interleukin-10 by Nucleocapsid Protein of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

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Keywords: *interleukin-10, nucleocapsid protein, PRRSV*

Introduction and Objectives

Porcine Reproductive and Respira-tory Syndrome Virus (PRRSV) causes economic loss in the pig industry worldwide including Thailand. Many studies indicate that PRRSV can increase IL-10 production in pigs during the early infection period (1), during the same period PRRSV also expresses high quantities of the nucleocapsid protein (N-protein) (2). This information suggests that N-protein might induce IL-10 production in pigs. In this study, the effect of N-protein on IL-10 production in porcine PBMC was investigated.

Materials and Methods

The ORF7 specific primers were designed for cloning the N-protein gene (ORF7, native conformation) and truncated N-protein (ORF7t, linear conformation) from the Thai isolate US-PRRSV (01NP1). The PCR products were cloned into pGEM?-T easy vector (Promega, USA). Subsequently ORF7 and ORF7t genes were subcloned into pQE31 vector (Qiagen, German) as a fusion protein with 6x-Histidine (6x-His) and expressed in the Escherichia coli strain M15.

Porcine peripheral blood mononuclear cells (PBMC) were isolated from the heparinized blood of PRRSV-seronegative pigs by density gradient centrifugation. The PBMC were co-cultured with the 5 μg/ml purified recombinant N-protein (ORF7) and truncated N-protein (ORF7t) for 48 hr. Negative control groups included either the PBMC cultured with elution buffer or protein expressed from pQE31. The positive control was the PBMC cultured with PRRSV. The expressions of IL-10 genes were analysed by semi-quantitative RT-mPCR (3). The intracellular expression of IL-10 was analyzed by flow cytometry (4).

Results and Discussion

N-protein (ORF7) induced IL-10 gene expression and increased the numbers of IL-10+ cells in the PBMC culture. However, truncated N-protein (ORF7t) could not induce IL-10 gene expression nor increase the number of IL-10+ cells (Figs. 1 & 2).

The results suggest that, PRRSV N-protein can induce IL-10 production in the PBMC and the conformation of N-protein plays significant role in induction of IL-10. This study identified the novel immunomodulatory mechanism of PRRSV.

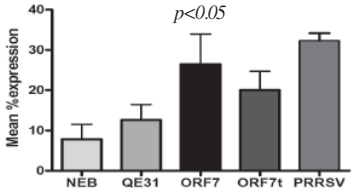

Figure 1. Mean percent expression of the IL-10 gene by the PBMC cultured with N-protein (ORF7) and truncated N-protein (ORF7t) (N=3). $p<0.05$ (ANOVA follow by the Tukey method)

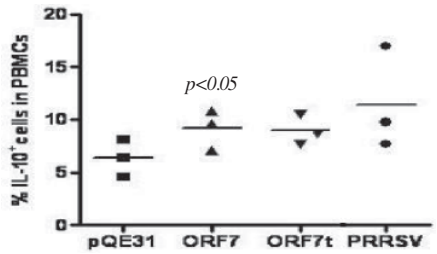

Figure 2. Total IL-10$^+$cells in PBMC, cultured with N-protein (ORF7) and truncated N-protein (ORF7t) (N=3). $p<0.05$ (ANOVA follow by Tukey method)

Acknowledgements

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Reference

1. Suradhat, S. and Thanawongnuwech, R. 2003. J. Gen. Virol. 84: 2755-2760.
2. Dea, S. et.al. 2000. Arch Virol. 145: 659-688
3. Suradhat, S et.al 2003. J. Gen. Virol. 84: 453-459.
4. Chokeshai-usaha, K et.al 2008. Proc. 20th IPVS Congress. P.407

P6 Comparison of the Developmental Ability and Quality of Cat IVF Embryos Produced from Fresh and Frozen-thawed Semen

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Keywords: *cat, embryos, fresh semen, frozen-thawed semen, IVF*

Introduction and Objective

In vitro fertilization (IVF) has been a useful technique for the conservation of endangered felid species that can be done with fresh and frozen semen. The objective of this study was to compare the developmental ability and quality of cat IVF embryos produced from fresh and frozen semen that will provide a basic knowledge to be applied in wild felids.

Materials and Methods

Oocytes were obtained from cat ovaries after ovariohysterectomy. *In vitro* embryo production technique followed according to Thongphakdee et al., 2007. Developmental ability of IVF embryos produced from fresh (n=132) and frozen semen (n=161) was compared. The number of total cells and the inner cell mass (ICM) of blastocyst reflecting embryo quality was assessed by differential cell staining. A statistical analysis of the development of embryos produced from fresh and frozen semen was tested by Chi-square. The average of the total cells and ICM numbers of blastocyst produced from fresh and frozen semen was tested by ANOVA.

Results and Discussion

The percentage of cleaved embryos produced from fresh semen was higher than that derived from frozen semen (68.39 vs 51.6%; $p<0.05$). However, Blastocyst formation success was not different between embryos produced from fresh and frozen semen (61.36 vs 60.87%; $p>0.05$). Numbers of ICM and total cell of blastocyst produced from fresh and frozen semen were no different ($p>0.05$), with 25.92±13.37 vs. 22.43±9.29 and 87.32±37.55 vs. 82.64±29.96 (cell/blastocyst), respectively. Observing embryos produced from frozen semen it was found that the percentage of cleaved at 24-, 48- and 72 h post-fertilization (pf) was 30.9, 42.3 and 49.1%, respectively. Thereafter, those embryos developed to blastocysts at day 5 pf and hatched blastocyst at day 6 pf.

The developmental ability of IVF embryos achieving blastocyst was similar whether fresh or frozen semen was used. The quality of embryos was not significantly different between those produced from fresh and frozen semen. In conclusion, fresh and frozen semen has the same ability to produce cat IVF embryos.

Acknowledgment

The study was financially supported by the Zoological Park Organization of HM the King, The Commission on Higher Education PhD sandwich program of the Thai government and the Reproductive Biological Science Research Unit, Chulalongkorn University.

References

1. Thongphakdee, A. et al. 2007. Proc. 33rd TVMA, Bangkok, 31 Oct.-2 Nov, p 49-50.
2. Tsujioka, T. et al. 2008. Reprod. Dom. Anim. 43(3): 323-327.

P7 Variation of the NSP2 Gene of PRRSV in Thailand

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Keywords: *NSP2 gene, pig, PRRSV*

Introduction and Objectives

Porcine reproductive and respiratory syndrome virus (PRRSV) is the cause of porcine reproductive and respiratory syndrome (PRRS), an economically important swine disease. The genetics of PRRSV are heterogeneous, especially in the nonstructural protein (nsp) 2-coding regions. PRRSV isolates with deletions in the nsp2 regions have been reported in some countries and some have been reported to be highly pathogenic, such as MN184 (USA) (1) and SY0608 (China) (20. However, the significance of the nsp2 genetic variation has not yet been elucidated. In this study, we determined full and partial nsp2 nucleotide sequences of Thai isolates of PRRSV and we demonstrate that some of these had nucleotide deletions in the nsp2-coding region.

Materials and Methods

The Thai PRRSV isolates in this study were provided by Chulalongkorn Veterinary Diagnostic Laboratory. Viral RNA was extracted using NucleoSpin® RNA Virus (Macherey-Nagel) and reverse transcribed using OmniScript RT kit (Qiagen). PCR was done using GoTaq® Green Master Mix (Promega) and nsp2-specific primers. PCR products were cloned into pGEM-T Easy vectors (Promega). Recombinant clones were sequenced using Automated DNA Sequencer. Sequences were analyzed using the Clustal W program in MEGA 4.0 (3).

Results and Discussion

One complete nsp2 sequence of the Thai isolates (08RB1) and two partial sequences (07NP4 and L119) were compared with that of VR2332, the prototypic strain, MN184 and SY0608, which have been shown to contain nucleotide deletions in the nsp2 regions and are reported to be highly pathogenic. In this study, these three Thai isolates were shown to contain deletion in the nsp2 region, compared to the VR2332 strain. The sequence analysis revealed that none of these deletion patterns were exactly the same as the previous reported isolates but more or less similar to the MN184 and HB-2 (china) isolates (Table 1 and data not shown).

In this study, we investigated the variation of the nsp2 gene of PRRSV in Thailand. We demonstrated that some of the Thai isolates also had nucleotide deletions in the nsp2 regions. The complete nsp2 sequence analysis of the 08RB1 isolate demonstrated the 08RB1 isolate had a 294-base (98-aa) discontinuous deletion, compared to the VR2332 strain, becoming the shortest nsp2 among Thai isolates that have been previously reported. It is interesting that whether or not? these Thai isolates, with deletion patterns were similar to that of the MN184, they were also highly pathogenic.

Table 1 Genetics profiles of PRRSV isolates

Isolates	Deletion	
	aa size	Possible aa position in nsp2 of VR2332
MN184	131	323-433, 482, 495-513
08RB1	98	332-428, 469
07NP4	47	328-366, 466-474
L119	97	332-428

References

1. Han et al. 2006. Virus Res. 122:175-82.
2. Li et al. 2007. Vet J. 174:577-84.
3. Tamura et al. 2007. Mol Biol Evol. 24:1596-99.

P8 Multiple Myeloma in a Dog

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Keywords: *dog, immunohistochemistry, multiple myeloma*

Introduction and Objectives

Multiple myeloma (MM) or plasma cell myeloma is a rare malignant tumor of well-differentiated B cell lymphocytes typically originating from the bone marrow that is associated with excessive secretions (1). This tumor secretes immunoglobulin molecules, indicating monoclonality, such as IgG, IgM, or IgA (2). The purposes of this study are to investigate a dog that clinically severe and was killed one day later following the result of the clinical diagnosis being aortic thromboembolism (ATE).

Materials and Methods

An 8-year-old, female Golden Retriever dog had severe acute clinical signs in both hind limbs, weakness, pulselessness, and cold extremities. Hematologic evaluation showed normal RBCs with thrombocytopenia, neutrophilia shift to the left and increasing serum ALP. Necropsy was performed and the affected organs were removed for routine histopathology. Sections were stained using polyclonal antibody against IgG and IgM (DAKO®, Denmark; 1:200) and monoclonal antibody against CD3 and CD20 (DAKO®, Denmark; 1:100), respectively.

Results and Discussion

The abdominal aorta revealed aortic thrombi size 1 cm, occluded in the distal aorta with an encapsulated multinodular mass (size 8 cm.), that was firm and creamy-yellowish on the cut surface and located beside the aorta. (Figure 1)

Histopathology revealed the tumor mass to be composed of pleomorphic discrete large round tumor cells with eccentric nuclei. The cytoplasm was abundant in perinuclear area. Some tumor cells were irregular with many multinucleated giant cells (H&E). Immunohistochemical staining showed strongly positive for IgG, IgM and slightly positive CD20 in some areas of the tumor mass but negative for CD3. (Figure 2)

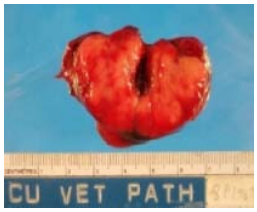

Figure1 Aortic thrombi occluded in the distal aorta firm and creamy-yellowish on the cut surface

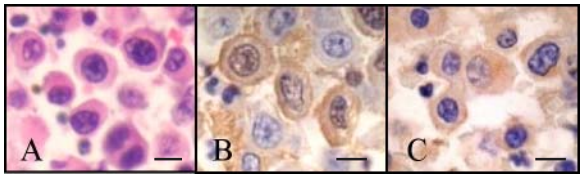

Figure 2 H&E staining (A) and Immunohistochemical staining showed positive for IgG (B) and CD20 (C). (Bar= 10μm)

Multiple myeloma was confirmed by the characteristic histopathological findings and immunohistochemical staining. This was associated with an excessive secretion of immunoglobulin and negative staining of CD3, surface glycoprotein on lymphocytes, as important marker for malignant lymphoma. The expression of CD20 by MM cells was heterogeneous, which could be detected only in 13-22% of MM cells and serve as malignancy and progression of MM (3). This tumorís secreting various paraproteins will cause hyperviscosity and secondarily to decreasing numbers of platelets; their function and sludging of blood that was the cause of death in this case.

References

1. Silva, P. et al. 2008. Braz. J. Vet. Pathol. 1(1): 21-24.
2. Kameyama, M. et al. 2003. J. Vet. Diagn. Invest. 15: 166-169.
3. Kapoor, P. et al. 2008. Brt. J. Haematology 141: 135-148.

P9 Effect of Equex STM Paste on Goat Semem Cryopreservation

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Keywords: *cryopreservation, Equex STM paste, extender, goat, semen*

Introduction and Objectives

addition of a detergent, Equex STM paste (Equex), in semen extender improves the post thaw quality of spermatozoa in many species (1), but a few reports in goat (2). This study aimed to evaluate the effect of Equex in semen extenders on goat semen quality after cryopreservation.

Material and Methods

Semen samples were obtained from three mature bucks of different breeds (Anglo-Nubian; A, Boer; B and Saanen; C) using an artificial vagina. The semen was evaluated as individual and pooled samples. The semen volume, sperm concentration, mass movement, motility, morphology, viability, membrane and acrosome integrity were evaluated. The semen samples showing >60% motility were submitted for cryopreservation. After removing seminal plasma, sperm pellet was diluted with a freezing medium composed of 14% glycerol + 10%egg yolk Tris-citric-fructose (v/v) [3] with or without 1% Equex (E+ or E-). The samples was equilibrated at 4°C for 4 hrs, cooled to -120°C for 10 min, and then frozen at -196°C (4). The semen was thawed at 37°C for 30 sec and the quality of the frozen-thawed semen was evaluated. The differences between the two groups were tested by an independent t-test (SPSS ver. 11.5)

Results and Discussion

The sperm motility of all samples in E+ were greater than E- ($p<0.05$). The percentage of normal tail of B, C and pooled semen were also higher in E+ group ($p<0.05$). In addition, sperm viability, the percentage of normal head and acrosome-intact spermatozoa of all samples in group E+ tend to have a higher values but not significantly different. However, no differences in the intact plasma membrane were observed in all groups.

Equex improved post-thawed semen quality in goat. The active compound in Equex is sodium dodecyl sulphate which might be exerted by enhancing the incorporation of egg yolk lipoproteins into sperm membrane (4). Moreover, it increases the semen qualities by acting as a surfactant to stabilize the cell membranes and to protect spermatozoa from the toxic effects of glycerol (5). However, addition of Equex in this experiment has no beneficial effect for the plasma membrane integrity similar to the studies in cats (6) and horses (5).

Acknowledgment

This work was supported by The Innovation Center for Veterinary Science, Faculty of Veterinary Science, Chulalongkorn University, Grant no. nuclear 01/52 and NA is granted by RGJ-PhD-industrial link program, Thailand Research Fund.

References

1. Ponglowhapan, S. and Chatdarong, K. 2008. Theriogenology. 69: 666-672.
2. Aboagla, E. and Terada, T. 2004. Theriogenology. 62: 1160-1172.
3. Chang et al. 2006. Reprod. Dom. Anim. 41: 55-60.
4. Arriola, J. and Foote, R.H. 1987. J. Dairy Sci. 70: 1664-1670.
5. Jimenez, C.F. 1987. Theriogenology. 28: 773-782.
6. Axnér et al. 2004. Anim. Reprod. Sci. 84: 179-191.

P10 Prevalence of Vancomycin-resistant Enterococci (VRE) in Companion Dogs and Cats in Thailand.

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Keywords: *cat, dog, enterococci, resistant, vancomycin,*

Introduction and Objectives

There is evidence of VRE related to avoparcin used in food-animals. However, VRE data in companion animals is lacking. Therefore, VRE prevalence and its antimicrobial resistance patterns in dogs and cats in Thailand has been studied.

Materials and Methods

Selective media containing 6 μg of vancomycin per mL was used for VRE screening. During 2003-2004; 530, 324, and 330 fecal samples from dogs and cats were randomly collected from the Small Animal Hospital at the College of Veterinary Medicine in Chulalongkorn University, Khon Kaen University and Chiang Mai University, respectively. An antimicrobial susceptibility test was performed using the agar dilution method.

Results and Discussion

The prevalence of Vancomycin intermediate-resistant Enterococci or VIRE (MIC = 8-16 μg/mL) and VRE (MIC ≥ 32 μg/mL) is shown in Figure 1. The majority of *Enterococcus* sp. was *E.faecium* (56.6 and 38.6% in dogs and cats, respectively). While *E.gallinarum* was 24.5 and 31.8%; and *E.faecalis* was11.3 and 18.2% in dogs and cats, respectively. Phenotype classification of VRE using glycopeptide-resistant patterns revealed VanA type 2% (2 strains of *E. faecium* and 1 strain of *E.faecalis*) and VanB type 1.3% (2 strains of *E.faecium*). Antimicrobial-resistant patterns of other antimicrobials tested for VRE are shown in Figure 2.

Since dogs and cats, have never been treated or directly exposed with glycopeptides and/or tylosin, there might be a preliminary conclusion that VRE is colonized in dogs and cats via food of animal origin or from the environment. The results indicate that VRE-colonization in dogs and cats could be related to humans and the community. Therefore, good sanitation practices should be followed with dogs and cats, as well as their litters in public.

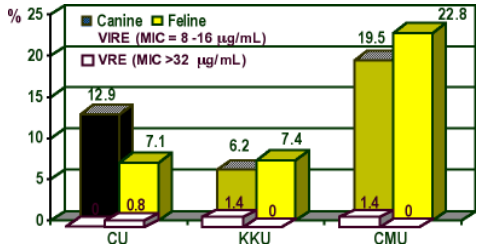

Figure 1 Prevalence of VIRE and VRE isolated from dogs and cats at Animal Hospitals in CU, KKU and CMU.

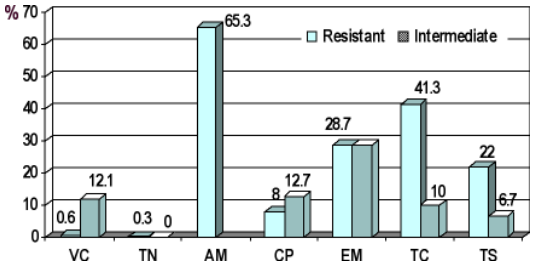

Figure 2 Antimicrobial resistant patterns of VRE isolated from dogs and cats at Animal Hospitals in CU, KKU and CMU. (vancomycin: VN, teicoplanin: TP, ampicillin: AM, chloramphenicol: CP, erythromycin: EM and tylosin: TS).

Acknowledgments

This research was supported by the Ratchadapiseksompoj Research Fund, Chulalongkorn University.

References

1. Aarestrup, F.M. et al. 2001 Antimicrob Agents Chemother 45(7): 2054-2059.
2. CLSI (transformed NCCLS ,1999)., 2008

P11 Prevalence and Van Gene of Vancomycin-Resistant Enterococci Isolated from Ark Shell *(Arca granulosa)* in Thailand

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Keywords: *antimicrobial drug resistance, polymerase chain reaction (PCR), VRE,*

Introduction and Objectives

The prevalence of vancomycin resistant enterococci (VRE) in Thailand has been already reported in farm animals, food of animal origin, domesticated animals and humans. However, VRE in the environment has not been studied in Thailand. Therefore this study used ark shells which had been cultivated from in Gulf of Thailand as biological markers of VRE in the environment.

Materials and Methods

This study was conducted from 2005-2006. Ark shell samples were pooled with 25 grams of each sample and added to a 225 ml PBS buffer. After homogenizing by stomacher, one mL of homogenized was add into 9 ml of KF broth. Samples were screened for VRE by bile esculin azide agar (BEA agar) containing 6 μg of vancomycin per mL. An antimicrobial susceptibility test was performed using the agar dilution method for vancomycin (VN), ampicillin (AP), cholramphenicol (CHPC), erythromycin (ET), tetracycline (TE), tylosin (TS) and E-test for teicoplanin (TP).

Results and Discussion

Pooled ark shell samples were found to have 26 isolates (4.3%) which were classified as *E. faecium* 15 isolates (57.7%), *E. faecalis* 6 isolates (23.1%), *E. gallinarum* 3 isolates(11.5%) and *E. casseliflavus* 2 isolates (7.7%). All of the VRE isolated from the pooled ark shell samples were found to have low level resistant to VN and sensitive to TP. E.faecium 15 isolates were resistant to AP, CHPC, TE and TS 13.3% and resistant to ET 33.3%. *E. faecalis* 6 isolates were susceptible to AP and CHPC and resistant to ET, TE and TS 16.7%. *E. gallinarum* were susceptible to AP and resistant to CHPC and TS 33.3% and resistant in ET and TE 66.7% and one from two of *E. casseliflvus* was resistant to ET only.

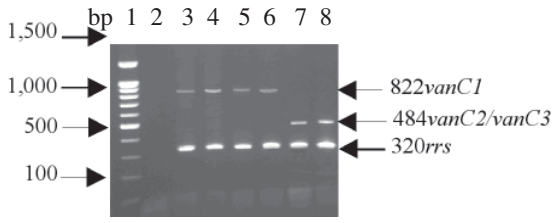

Figure 1 The results of multiple PCR assays that contained the three primer sets

Detection of the van gene from all of low level resistance VRE by polymerase chain reaction (PCR) found gene *vanC1* in all of *E. gallinarum* isolates and vanC2/C3 in all of *E. casseliflavus* isolates. A low prevalence of VRE was found in this study which show a low level resistance to vancomycin and a susceptibilty to teicoplanin. Therefore VRE should not be a public health threat in Thailand.

Acknowledgements

This research was supported by Thai Government Research Fund.

References

1. Harbarth, S. et al. 2002 Antimicrob Agents Chemother.46(6): 1619-1628.
2. Wegener, H.C. et al. 1997 Int. J. Food Microbiol. : 57-66.
3. CLSI (transformed NCCLS 1999), 2008

P12 Blood Parasites of the Rice Field Frog, *Hoplobatrachus rugulosus* (Wiegmann, 1835), from Wang Nam Yen district, Sra-kaew Province, Thailand

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Keywords: *blood parasite, Hepatozoon* sp., *Hoplogatrachus rugulosus, Lankesterella* sp., *Trypanosoma* sp.,

Introduction and Objectives

Studies on anuran parasites have been reported in several geographical regions and a wide variety of intra- and extracellular blood parasites have been observed (1, 4, 5). In Thailand, Amphibians parasites were preliminary surveyed and recorded. The major observed parasites were blood parasites such as Trypanosomatids and Haemogregarines and Helminthes such as *Cosmocerca* sp. (2, 3). The rice field frog, (*Hoplobatrachus rugulosus*, Wiegmann, 1835), is a frog native to Thailand and has high economic importance. The purpose of this investigation was to survey the natural prevalence of the blood parasites of rice field frog occurring at Wang Nam Yen district, Sra-kaew province, Thailand.

Materials and Methods

Between July and October 2007, 140 rice field frogs from Wang Nam Yen district were examined. The frogs were collected by local commercial vendors. Blood was obtained from the heart and subsequently made blood smear slide. Impression smear slides were also made from the frogís liver, lung, spleen and kidneys. These slides were air dried, fixed with absolute methanol and stained with Giemsa (1:10 in phosphate buffer pH 7.2) for 30 minutes. Each film smear was observed under a microscopic for blood parasites. The photographs were taken by Olympus digital camera model C-5050 in a Olympus compound light microscope model CH30.

Results and Discussion

The blood parasites, *Trypanosoma* sp. (fig. 1-6), *Hepatozoon* sp. (fig. 7,8) and *Lankesterella* sp. (fig. 9) were observed in the blood of *H. rugulosus*. The prevalence of these parasites is shown in Table I.

H. rugulosus showed a high natural prevalence of blood parasite infection which agrees with the earlier study (3), except for Rickettsia which was not found in the frogs captured

in Wang Nam Yen district. This can be attributed to the habitat of the frogs. *H. rugulosus* is an aquatic frog which mainly lived in water with a high incidence of infection by parasites (5). The obtained data is useful for the health monitoring of natural frogs.

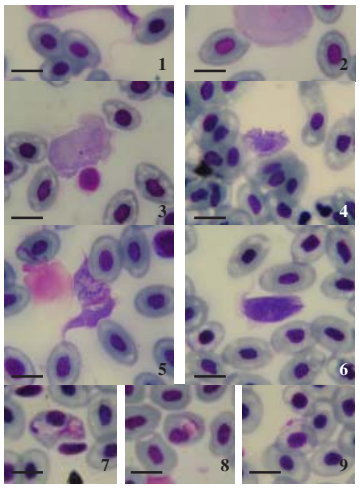
Acknowledgements

This study was supported by the Center of Excellence in Biodiversity and the Amphibian and Reptile Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University.

Table 1 Parasitic percent age prevalence of *H.rugulosus* from Wang Nam Yen district, Thailand

Host	***Hoplobatrachus rugulosus***
Parasite	Percent prevalence
T. sp.[a] (a)	12.1(17)[d]
T. sp. (b)	32.1(45)
T. sp. (c)	4.3(6)
T. sp. (d)	7.9(11)
T. sp. (e)	1.4(2)
T. sp. (f)	2.1(3)
H. sp.[b] (a)	20.0(28)
H. sp. (b)	3.6(5)
L. sp.c	16.4(23)

[a]*Trypanosoma* sp., [b]*Hepatozoon* sp., [c]*Lankesterella* sp., [d]Sample number were infected



Figures 1-9. Photomicrographs of the blood parasites of *Hoplobatrachus rugulosus* 1. *Trypanosoma* sp. (a) 2. *Trypanosoma* sp. (b) 3. *Trypanosoma* sp. (c) 4. Trypanosoma sp. (d) 5. *Trypanosoma* sp. (e) 6. *Trypanosoma* sp. (f) 7. Mature gamont of *Hepatozoon* sp. (a) in red blood cell. 8. Gamont of *Hepatozoon* sp. (b) in red blood cell. 9. Extracellular sporozoite of *Lankesterella* sp. Bar = 10 μm

References

1. Barta, J.R. and Desser, S. S. 1984. J. Wildlife Dis. 20(3):180-189.
2. Chutmongkonkul, M. and Pariyanonth, P. 2005. 5th World Congress of Herpetology, Stellenbosch, South Africa. p.125.
3. Chutmongkonkul, M. and Pariyanonth, P. 2007. 14th Proceedings Association of Reptilian and Amphibians Veterinarians, New Orleans, Lousiana, USA p. 118.
4. Desser, S. S. 2001. J. Parasitol. 87(1):.152-160.
5. Werner, J. K. 1993. J. Parasitol. 79(3): 356-363.

P13 Redistribution of Cytoskeleton and Chromatin Configurations during Early Embryo Development in Swamp Buffalo *(Bubalus bubalis)*

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Keywords: *cytoskeleton, embryo development, swamp buffalo*

Introduction and Objectives

Cytoskeleton elements (actin microfilaments and microtubules) play a central role in early embryo development, especially during the syngamy of two haploid pronuclei resulting in a diploid zygote nucleus. In mice, the fertilization process depends on maternal-centrosome inheritance, while a number of studies in several mammal species reveal only the paternal inheritance (1). These events, however, have not yet been studied in swamp buffalo. We aimed to examine the redistribution of cytoskeleton and chromatin patterns during early embryo development in swamp buffalo.

Materials and Methods

A total of 480 immature oocytes were aspirated from ovaries obtained from local slaughterhouses. After 22 h of *in vitro* maturation, the oocytes were fertilized in vitro using "swim-up" frozen-thawed sperm in TALP medium. All presumptive zygotes were fixed at various time points (6, 12, 18, 24, 30 or 48 h) after *in vitro* fertilization. Immunofluorescent labelling of microtubules, actin microfilaments and chromatin was performed using monoclonal-α-tubulin-TRIT C Alexa 488 phalloidin and DAPI, respectively. The zygotes were examined using an epifluorescent microscope.

Results and Discussion

The fertilization rate was 49.7% (139/301). At 12 h post IVF, the pronucleus formation (72.2%) was observed and this was increased to 97.6% at h 18 (Fig. 1A & 1C). A syngamy of male and female pronuclei was observed by h 24 (48.8%, 21/43) (Fig. 1F). The first cleavages occurred by h 30 (52.4%, 11/21) (Fig. 1G & 1I). At h 48, the percentage of cleavage embryos was 79.3% (23/29). During the pronuclear stage, both of the two pronuclei continued to enlarge and the sperm astral microtubules, originating from the sperm neck region, also increased in size (Fig. 1B & 1I). When the pronuclei were in apposition before syngamy, a dense array of microtubules was present between the pronuclei (Fig. 1D & 1E). As the cell cycle progress, actin microfilaments formed a "furrow" between the dividing cell (Fig. 1H). Cell cytoskeleton plays an essential role during fertilization and early embryo development in swamp buffalo. Centrosomal materials, originating from the sperm, are actively involved in the migration and the apposition of the two pronuclei, which is similar to cattle (2).

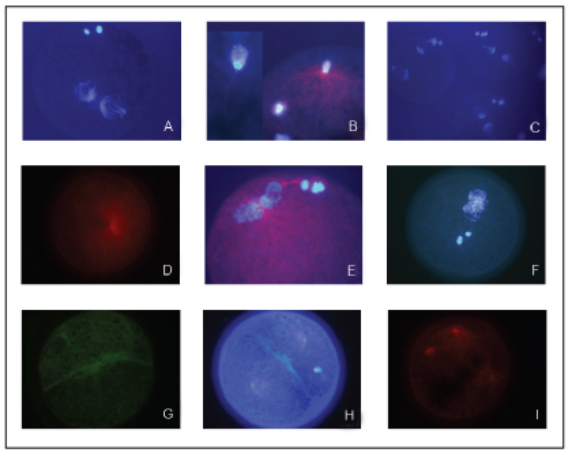

Figure 1. Images of buffalo oocytes after staining with monoclonal-α-tubulin-TRIT C to demonstrate microtubules, 488 phalloidin to identify microfilaments and DAPI to label the chromatin.

References

1. Sathananthan, A.H. 1998. J. Assist. Reprod. Gen. 15: 129-139.
2. Long, C.R. et al. 1993. Mol. Reprod. Dev. 36: 23-32.

P14 *In vitro* Fertilization of Porcine Oocytes by Frozen-thawed Semen: Effect of Sperm: oocyte Ratio

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Keywords: *in vitro fertilization, porcine embryo, frozen semen*

Introduction and Objectives

Incomplete cytoplasmic maturation of porcine oocytes matured *in vitro* increases the incidence of polyspermy that occurs more frequently in pig than in other species (1). This limits the biomedical and embryonic stem cells (ESCs) research. (2). A high concentration of spermatozoa leads to the enhanced frequency of polyspermic penetration (3). This study aimed to examine the effect of sperm:oocyte ratio on the developmental competence and quality of porcine embryos using frozen boar semen.

Materials and Method

Compacted cumulus-oocyte complexes (COCs, n = 831) were collected from porcine ovaries and then cultured in IVM medium. After 44 h of culture, groups of 30-50 COCs were maintained at 38.5°C in 500 µl of IVF medium at a humidified condition of 5% CO_2 in air. Frozen-thawed semen from a boar was used for IVF. Three different ratios of sperm:oocyte (1,000:1, 2,000:1, and 4,000:1) were performed. After the co-incubation of sperm and oocytes for 6 h, cumulus cells were removed. The presumptive zygotes were subsequently cultured for an additional 7 days. The rates of normal fertilization, cleavage and blastocyst formation were examined at 20 h, 48 h and 144 h post IVF, respectively. The quality of blastocyst was assessed by means of the cell numbers of the inner cell mass (ICM) and trophectoderm (TE). The difference among the groups was tested by ANOVA.

Results and Discussion

As shown in table 1, the penetration rate did not significantly differ among experimental groups. An increase of sperm:oocyte ratios from 2,000 to 4,000 sperm per oocyte significantly reduced the rate of monospermy compared with 1,000 sperm:oocyte group (Table 1). At 48 h after IVF, cleavage rates were not significantly different among the groups, whereas blastocyst rates increased significantly in the group fertilized with 1,000 sperm:oocyte compared to those of 4,000 sperm:oocyte (29±1.8 vs 14±2.8%; mean±S.E.M, respectively; Fig. 1), however, blastocyst quality was not different in any group. A low sperm:oocyte ratio during IVF improves the proportions of monospermic embryos and blastocyst development, Increasing sperm numbers, however negatively affects the success of embryo production in pig.

Table 1 Effect of sperm:oocyte ratios during IVF of pig oocytes matured *in vitro*.

Ratio	oocytes examined (no.)	Percentage of oocytes Penetrated	Monospermic*	Efficiency** (%)
1,000:1	89	79.26±5.3	73.43±8.7[a]	56.51±4.7[a]
2,000:1	79	90.23±2.53	48.07±6.0[b,c]	43.96±6.9[a,b]
4,000:1	85	93.46±3.7	31.51±4.9[c]	29.36±4.8[b]

*Percentage of the number of monospermic oocytes/total of fertilized oocytes;

**percentage of the number of monospermic oocytes/total number of examined oocytes. a,b,c $p < 0.05$.

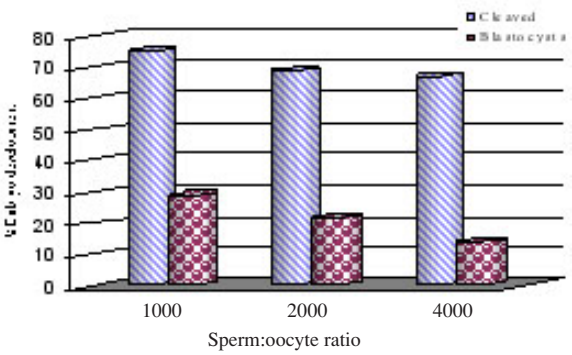

Figure 1 Effect of sperm:oocyte ratios on percentage of embryo development after IVF using

References

1. Wang et al. 1997. J Reprod. Fertil . 111: 101-108.
2. Koo et al. 2005. Ani. Reprod. Sci. 90: 101-110.
3. Matas et al. 2003. Reproduction. 125: 133-134.

P15 Water Temperature at the 60th Anniversary Veterinary Science Building, Chulalongkorn University between 2004 and 2008

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Keywords: *aquaculture, aquatic animal health, stress, tap water, water quality, water temperature*

Introduction and Objectives

Water quality is important for human supply and aquatic animal culture. The Metropolitan Waterworks Authority and the Department of Fisheries have announced different guidelines for their own purposes (Table 1). Tap water is used to raise aquatic animals in laboratories of Chulalongkorn University. Therefore, water quality monitoring plays a major role in aquatic animal health.

Materials and Methods

Water from the indoor lab, unit of Aquatic Animal Medicine, 2nd floor, 60th Anniversary Veterinary Science Building, CU, was randomly sampled monthly in November-February from 2004 - 2008. Air temperature, water temperature and DO were checked up by Oxygen meter (YSI model 57, USA). The rest of the water parameters employed commercial test kits (Aqua-VBC, Thailand).

Table 1 Acceptable data for fresh water quality.

Parameters (unit)	Municipal Water[A]	Aquaculture[B]
Air temperature (At,°C)	ND	ND
Water temperature (Wt,°C)	ND	23.0-32.0
Dissolved Oxygen (DO, ppm)	ND	>3.0
pH	ND	5.0-9.0
Alkalinity (Alk, ppm)	ND	ND
Hardness (Hd, ppm)	ND	ND
Calcium (Ca, ppm)	ND	ND
Magnesium (Mg, ppm)	ND	ND
Ammonia(Amm, ppm)	ND	ND
Chlorine (Cl, ppm)	0.2	0.005
Copper (Cu, ppm)	2.0	0.02
Cyanide (CN, ppm)	0.07	ND
Iron (Fe, ppm)	0.3	0.3
Manganese (Mn, ppm)	0.4	ND
Nitrate (Nta, ppm)	50.0	ND
Nitrite (Nti, ppm)	3.0	ND
Phosphate (PO_4, ppm)	ND	ND

A: Metropolitan Waterworks Authority Bangkok Thailand, B: Department of Fisheries Bangkok Thailand, ND: No Data

Results and Discussion

Water temperature dropped from 27°C to 21°C because of air temperature (Table 2). Temperature is known to have a strong influence on aquatic animal health (1). Immunity, infectious diseases and stress are examples. Heater application, surplus vitamin C to the diet and 0.1% NaCl (2) to water is recommended for de-stressing at low temperature.

Table 2 Water quality from the indoor lab, 2nd floor, 60th Anniversary Veterinary Science Building, CU. (data shown as mean of 4 rep)

Parameters	04	05	06	07	08
At	28.7	28.2	28.5	27.5	23.2
Wt	27.5	26.7	27.0	26.7	21.3
DO	5.3	5.4	5.0	5.0	5.5
pH	7.5	7.5	7.3	7.3	7.8
Alk	132	87.2	73	85	109
Hd	136	114	110	110	85
Ca	ND	ND	70	100	100
Mg	ND	ND	8	10.5	0
Amm	0	0.1	0	0.1	0
Cl	0	0	0	0	0
Cu	ND	0	0	0	0
CN	ND	0	0	0	0
Fe	ND	0	0	0	0
Mn	ND	ND	0	0	0
Nta	2.5	5.2	0.6	8.2	0.7
Nti	0.01	0.2	0	0	0.1
PO_4	ND	0.1	0.9	0.9	1.1

ND: not done, 04: Nov2004-Feb2005, 05: Nov2005-Feb2006, 06: Nov2006-Feb2007, 07: Nov2007-Feb2008, 08: Nov2008-Dec2008 and data shown as mean of 2 replicates

Acknowledgements

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References

1. Langston, A.L. et al. 2002. Fish & Shellfish Immunology. 12: 61-76
2. Tsuzuki, M.Y. et al. 2001. Aquaculture. 200: 349-362.

P16 Histopathological and Autometallographic Tracing of Acute Mercury Toxicity in Tilapia *(Oreochromis niloticus)*

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Keywords: *autometallography, histopathology, mercury, tilapia*

Introduction and Objectives

Mercury is one of most toxic heavy metals which can contaminate natural water sources. Humans can be exposed to mercury by inhalation and ingestion and especially by the consumption of fish. (1) Therefore, it is most appropriate to use fish as environmental marker of mercury contamination. (2) The purpose of this study is to investigate acute mercury toxicity in tilapia *(Oreochromis niloticus)* via histopathological and autometallographic techniques.

Material and Methods

Tilapia *(Orechromis niloticus)* (9-13 cm; 25-50 g), were divided into eight experimental groups, in each group, twenty five tilapia were placed in 62.5-l glass aquaria filled with 50-l tapwater. Four of the groups were intraperitoneally injected with 0.5, 1, 2, and 5 μg/g (ppm) mercuric chloride ($HgCl_2$, Sigma-Aldrich) respectively and the rest of the aquaria were bathed with 0.5, 1, 2, and 5 μg/l (ppm) $HgCl_2$ respectively. There was also one control group. All fish were fed twice daily with commercially available food throughout the experimental period. Water in the aquaria were hanged every two days. Samples (gill, liver, spleen, intestine, brain, heart, and muscle tissues) were collected after euthanasia by hypothermia every three days until the 15th day of exposure. All samples were fixed with 10% buffered formalin, embedded in paraffin, sectioned at 6 μm, then stained with heamatoxylin and eosin (H&E) and the mercury visualized by autmometallographic method. (3) The results were observed under light microscope.

Results and Discussion

Mercuric chloride can produce remarkable histopathological changes in tilapia, especially in the kidneys, hepatopancreas and spleen. All animals were dead in 2 and 5 μg/l $HgCl_2$ bath groups on the first day of experiment. Severe tubulonephrosis; swelling of tubular epithelial cells with hyaline droplets and hyaline casts in tubular lumen, was observed on the first day. (day 0) Immature nephrons; regenerating tubular epithelium, were also found on days 6, 9, 12 and 15 of the experiment. The histological changes of liver were the regenerating of hepatocytes after 6 days. The hepatocytes were swellings with large nuclei and prominent nucleoli and a loss of cytoplasmic fat when compared with the control group. Mild pancreatic atrophy could also be found. There was a marked increasing of melanomacrophage centers (MMCs) in splenic parenchyma compared with the control group. MMCs were increased on days 3, 6, 9, 12 and 15 respectively. The kidney is a target organ of heavy metals, such as Hg, in species including mammals. (4) High concentrations of Hg compounds produce severe tubulonephrosis. Hepatocytes regenerate and loss of fat storage is involved in the hepatic detoxification of Hg. (5) Increasing of MMCs in spleen and head of kidney may involve an increase phagocytic activity due to tissue damage by Hg given that macrophages belong to the first line of protection of mercury intoxification. (3) Following autometallography development, silver-enhanced Hg grains are visualized as black granules in renal tubular epithelium, MMCs in spleen, in head of the kidney and in macrophages at pancreatic acini. From an autometallographic tracing of mercury, the trunk of the kidney and spleen of tilapia are major organs for mercury accumulation. The amount and location of mercury grains are different after observation between administration routes and doses. (3) The bath group demonstrated more mercury grains than the i.p. administration group and higher concentrations of $HgCl_2$ produced more grains than lower concentrations.

Acknowledgements

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References

1. WHO. 2003. Concise International Chemical Assessment Document. 50:1-32.
2. Pandey et al. 2005. Ecotoxicol Environ Saf 61: 1144-1154.
3. Loumbourdis and Danscher. 2004. Environ Pollut. 129 (4): 299-304.
4. Hansen and Danscher. 1995. Pharmacol. Toxicol. 77(3): 189-195.
5. Mela et al. 2007. Ecotocicol. Environ. Saf. 68(3): 426-435

P17 Effect of *Streptococcus uberis* Causing Intramammary Infection on Raw Milk Compositions

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Keyword: *intramammary infection, milk compositions Streptococcus uberis.*

Introduction and Objectives

Streptococcus uberis is responsible for clinical and subclinical bovine mastitis. Previous studies shown that 95 percentage of *Streptococcus uberis* infection is subclinical in lactating cows (1). The current mastitis control program effectively reduces contagious mastitis. However, environmental mastitis is becoming dominant pathogens of the mammary glands especially *Streptococcus uberis* (2-4). Mastitis affects the quality of milk via increase in the somatic cell counts and a decrease in lactose, fat and casein (5, 6). Previous study may refer to milk composition change due to subclinical mastitis (7), but no evidence of *Streptococcus uberis* intramammary infection (IMI) affecting milk compositions. This study investigated raw milk composition changes in *Streptococcus uberis* IMI.

Materials and Methods

Farms and animals: Eighteen small dairy holders with three hundred and four lactating cows in Nakhonpathom province, Thailand.

Sampling procedures: Milk samples from farms were collected every other month. Quarter foremilk samples from all udder quarters were obtained to identify subclinical mastitis using the California Mastitis Test (CMT). Milk from CMT positive cows was collected after milking.

Laboratory procedures: Post milking milk samples were obtained aseptic technique (8). Milk samples were determined for microbiological analyses (9). *Streptococcus uberis* were identified to the species level using API 20 Strep identification system (bioMerieux Vitek Inc., Hazelwood, MO, USA). Measurement of somatic cell counts in milk with Coulter Counters ZM® (10). Milk composition was determined by Milkoscan® model 133B.

Statistical analyses: Comparison within the same cow of somatic cell score and milk compositions including percentages of protein, fat, lactose, total solid and solid not fat between *Streptococcus uberis* infected quarters (n = 45 quarters) and healthy quarters (n = 45 quarters) by SAS® 8.0. (Mixed procedure). Results are presented as least-square (LS) means.

Table 1. Comparison of milk compositions (post milking samples) between Streptococcus uberis infected quarters and healthy quarters within the same cow.

Variable	LS means		SEM	T	P- value
	S. uberis	Healthy			
SCS*	7.24	4.13	0.27	-8.28	*<0.0001*
Protein	3.53	3.37	0.10	-2.01	*0.0509*
Lactose	3.89	4.32	0.09	3.41	*0.0015*
Fat	6.11	7.11	0.36	2.84	*0.0071*
SNF	8.14	8.40	0.14	1.97	*0.0560*
TS	14.24	15.51	0.37	3.22	*0.0026*
F/P	1.81	2.24	0.13	3.07	*0.0038*

*SCS = 3 + log2 (SCC/100,000) as describe by Anderson (11).

Results and Discussion

The average prevalence of *Streptococcus uberis* IMI in lactating cows in these small dairy holders was 40.90% during our study. Almost all *Streptococcus uberis* IMIs were subclinical mastitis (97.78%). The differences in SCS, percentages of protein, fat, lactose, total solid and solid non fat between *Streptococcus uberis* IMI quarters and healthy quarters were significant as shown in Table 1. *Streptococcus uberis* IMIs causing subclinical mastitis in this investigation was related to the changes of milk composition including elevated somatic cells and a decreasing in the percentage of lactose, fat, total solid and fat protein ratio (F/P). In Thailand, total solid is one of the importance parameters for raw milk quality and pricing. *Streptococcus uberis* causing mastitis at certain levels may cause economic loss due to not only production loss but also milk quality. The control and treatment strategies for *Streptococcus uberis* IMI need to be economically further evaluated.

References

1. Jayarao et al. 1999. J Vet. Med. 46: 433-442.
2. James. 1999. Vet. J. 157: 225-238.
3. Bradley. 2002. Vet. J. 164: 116-128.
4. Hillerton and Berry. 2003. Vet Clin North Am Food Anima Pract 19: 157-169.
5. Urech et al. 1999. J. Dairy Sci. 82: 2402-2411.
6. Pyörälä. 2003.Vet. Res. 34: 565-578.
7. Bruckmaier et al., 2004. Vet Med-Czech 8: 283-290.
8. Marshall. 1992. Standard Methods for The Examination of Dairy Products. 68-72., 334-338
9. Laboratory and Field Handbook on Bovine Mastitis. 1999. Natl mastitis counc.
10. Anderson. 1993. Vet Clin North Am Food Anima Pract 9: 579-581.

P18 *In vitro* Growth Effect of *Streptococcus uberis* Causing Subclinical Mastitis Isolates on Methylene Blue Reduction Test

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Keywords: *intramammary infection, isolate, growth, methylene blue reduction test Streptococcus uberis,*

Introduction and Objectives

Streptococcus uberis is one of the most important bacterial intramammary infections (IMI) in dairy cattle (1, 2). The prevalence of mastitis caused by this bacteria is in the range of 12-19% (3, 4). In Thailand, the prevalence of this pathogen is about 65% (5). The ranges of bacterial number shedding from *Staphylococcus aureus* IMIs were 1,000-2,000 cfu/ml (6) and from *Streptococcus uberis* IMIs were 30-66,000 cfu/ml (7). These shedding IMI pathogens can contaminate raw milk and be the initial bacterial number affecting milk quality during raw milk storage and transportation. The methylene blue reduction test (MBRT) is the crucial test for screening bacterial contamination, quality grading and pricing of raw milk at milk collecting centers in Thailand. In this investigation, we hypothesized that the initial bacterial number of contaminated *Streptococcus uberis* and variety of these isolates from IMI could differently alter growth itself and the result of the MBRT.

Materials and Methods

Bacterial isolates: In vitro experiment using nine *Streptococcus uberis* isolates from subclinical mastitis cases which were classified into three different profiles (I, II and III).

Laboratory analyses: Cultures of *Streptococcus uberis* isolates were prepared to 0.5 McFarland. Bacterial concentration of each isolate was prepared to 10^6, 10^5 and 10^4 cfu/ml in 10 ml of UHT milk. One milliliter of methylene blue thiocyanate solution was added into a test tube. Tubes were immediately incubated in water bath at 37°C. Decolorization of the MBRT was examined and recorded after the first 30 minutes and every hour after incubation until 8 hours. Every one hour, one milliliter of milk sample was taken and determined the number of bacteria determined using the standard plate count method (8). The result is presented in log colony forming unit (cfu/ml).

Data analysis: Descriptions of *Streptococcus uberis* growth and time of MBRT decolorization were compared among different API profiles.

Results and Discussion

Growth and time of MBRT decolorization *Streptococcus uberis* isolates are illustrated in Table 1. According to API profiles (I, II, and III), there were similar patterns of growth among different profiles. However, the initial numbers of bacteria and incubation period (hour) were related to the growth and color change of MBRT. Time of MBRT decolorization was dependent on the initial numbers of bacteria, except one isolate (no. 8). In addition, most of MBRT decolorizations occurred when the number of bacteria reached log 6.70-8.65 (I), log 7.18-8.86 (II) and log 7.52-8.82 cfu/ml (III). MBRT decolorizations (21/27) have occurred when numbers of bacteria above log 8.0. We found that the higher initial number of bacteria was the faster time of MBRT decolorization could appear. Our results indicated that contamination of *Streptococcus uberis* could be high as log 8.71 without any color change of MBRT (4 hrs. incubation). MBRT may have disadvantage on the measurement of *Streptococcus uberis* contamination in raw milk. In farm situations, the number of *Streptococcus uberis* IMI cows (9) and milk weight of IMI cows could be the crucial factors affecting the initial bacterial number of *Streptococcus uberis* in bulk tank milk. The variety of isolates at different initial concentrations gave similar growth results. However, only initial concentration affected MBRT.

Table 1. Growth of *Streptococcus uberis* IMI isolates and time of MBRT decolorization.

API profiles		Initial bacterial number	Time (hour)												
			0	1	2	3	3.3	4	4.3	5	5.3	6	6.3	7	7.3	8.0
(I) 7442710	1	10^6	6.66	6.71	7.41	8.20	-	8.71	8.65*	8.70	8.70	8.70	8.68	8.65	8.52	8.39
		10^5	5.66	5.83	6.54	7.25	-	8.16	8.88	8.91	8.85*	8.88	8.44	8.49	8.47	8.45
		10^4	4.64	4.82	5.36	6.26	-	7.00	7.24	7.69	7.93	8.17	8.43*	8.32	8.34	8.36
	2	10^6	5.54	6.34	7.26	7.82	8.09*	8.36	8.22	8.08	8.06	8.04	8.14	8.23	8.25	8.23
		10^5	4.87	5.34	6.40	6.78	6.87	7.20	7.37	7.58	7.78*	7.68	7.78	7.89	7.94	7.89
		10^4	3.62	4.58	5.26	5.59	5.83	6.28	6.54	6.79	7.02	7.26	7.71*	7.69	7.85	8.00
	3	10^6	5.36	5.75	6.42	6.81	-	7.50	-	7.93	8.10	8.28	8.58*	8.45	8.60	8.75
		10^5	4.29	4.72	5.37	5.99	-	6.36	-	7.04	7.13	7.22	7.46	7.71	8.08*	7.80
		10^4	3.24	3.74	4.26	4.79	-	5.48	-	6.04	6.22	6.40	6.46	6.53	6.78*	6.83
(II) 7442710	4	10^6	5.79	6.70	7.25	8.14	-	8.11	8.25*	8.27	8.49	8.71	8.70	8.69	8.63	8.56
		10^5	4.83	5.57	6.25	6.11	-	6.47	6.55	6.60	6.89	7.19	7.49	7.79	7.57	7.36*
		10^4	2.74	4.73	5.30	5.30	-	5.50	5.63	5.76	5.84	5.92	6.00	6.07	6.62	7.17*
	5	10^6	5.18	5.36	6.11	7.52	-	7.91	-	8.15*	8.27	8.30	8.16	8.38	8.46	8.53
		10^5	4.25	4.49	5.40	6.25	-	6.89	-	7.43	7.75	8.05	8.49*	8.35	8.57	8.79
		10^4	3.18	3.59	4.25	5.03	-	5.81	-	6.53	6.88	7.25	7.65	8.07	8.42*	8.43
	6	10^6	5.86	6.37	7.39	8.09	-	8.41	8.78*	8.87	8.89	8.93	8.89	8.87	8.87	8.87
		10^5	4.85	5.65	6.48	7.23	-	7.75	8.14	8.54	8.78*	8.79	8.80	8.80	8.83	8.86
		10^4	3.76	4.49	5.58	6.07	-	6.79	7.28	7.69	7.97	8.20	8.41	8.58	8.86*	8.95
(III) 7545715	7	10^6	5.64	6.41	6.99	7.39	-	8.21	8.51	8.82*	8.83	8.85	8.99	9.14	9.14	9.14
		10^5	5.00	5.46	6.12	6.52	-	7.22	7.45	7.69	7.96	8.24	8.46*	8.79	8.91	9.04
		10^4	3.97	4.54	5.01	5.60	-	6.18	6.46	6.73	6.94	7.14	7.61	8.08	8.21	8.34*
	8	10^6	5.69	6.36	7.08	7.60	-	8.04	8.14	8.23	8.19	8.15	8.07*	8.23	8.25	8.23
		10^5	4.83	5.36	6.15	6.62	-	7.11	7.30	7.48	7.70	7.93	8.08*	8.18	8.23	8.28
		10^4	3.64	4.26	5.00	5.36	-	5.89	6.10	6.30	6.60	6.90	7.52*	7.48	7.71	7.95
	9	10^6	5.96	5.83	6.47	7.01	-	7.93	-	8.40	8.68*	8.84	8.93	8.87	8.86	8.89
		10^5	4.87	4.86	5.51	6.30	-	7.00	-	7.75	8.06	8.30	8.52*	8.47	8.60	8.72
		10^4	3.74	3.87	4.54	5.41	-	6.01	-	6.58	7.01	7.45	7.71	7.97	8.32*	8.75

*Time of Methylene blue decolorization

References

1. Leigh. 1999. Vet J 157: 225-238.
2. Zadok. 2002. Dissertation University of Utrecht. 237 pp.
3. Jayarao et al., 1999. J Vet. Med. 46: 433-442.
4. Barrett et al., 2005. Irish Vet. J. 58: 333-337.
5. Ajariyakhajorn. 2005. Proc. 4th Chula. Uni. Vet. Sci. Con. : 91.
6. Sears et al., 1990. J. Dairy Sci. 73: 2785-2789.
7. Rerk-u-suke. 2008. Dissertation University of Chulalongkorn.
8. Marshall. 1992. Standard methods for the examination of dairy products. 16th ed. 213-225.
9. Dogan and Boor. 2004. J. Dairy Sci. 87: 813-815.

P19 Prevalence and Antimicrobial Resistance of *Staphylococcus intermedius* Group (SIG) Isolated from Skin Lesions of Dogs in Thailand

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Keywords: *antimicrobial resistance, dogs, skin lesions, Staphylococcus intermedius Group (SIG)*

Introduction and Objectives

Several documents report that the *Staphylococcus intermedius* group (SIG), which consist of *S. intermedius*, *S. pseudintermedius*, and *S. delphini*, are the main pathogens causing bacterial skin diseases in dogs (3-5). Most of the documents show that *S. intermedius* isolated from dogs are resistant to beta-lactams, chloramphenicol, aminoglycosides, tetracyclines and fluoroquinolones but not found to be resistant to cephalosporins, amoxycillin-clavulanate oxacillin and sulfa-trimethoprim (3, 4, 6). Cephalosporins are the drug of choices for treatment of bacterial skin diseases of dogs in Thailand. However, non-prudent using of antimicrobial drugs may develop antimicrobial resistant bacteria in animal and can transfer the resistance to other bacteria in human (4). The objective of this research is to study the prevalence and antimicrobial resistance of SIG isolated from skin lesions of dogs in Thailand. This information will be useful for Thai Veterinary practitioners for the control and treatment of SIG infected skin of dogs.

Materials and Methods

Isolation of bacteria from samples taken from skin lesions of dogs entering the small animal veterinary teaching hospital, Chulalongkorn University, Thailand in year 2005-2006. The isolated bacteria was identified based on the phenotypic properties. However, the method could not identify species of SIG (1, 5). Subsequently, calculation of the prevalence of SIG was done. The antimicrobial susceptibility testing of SIG to 14 antimicrobial drugs (cefotaxime sodium, ceftazidime, ceftriazone, aztreonam, cefpodoxime, cephalexin, enrofloxacin, ciprofloxacin, ampicillin, amoxycillin, sulfamethoxazolñtrimetroprim, doxycycline, gentamicin, chloramphenicol) was performed by standard disc diffusion method (2).

Results and Discussion

Six genus of bacteria (n=54) were isolated and identified from dog skin lesions. The most common isolated bacteria was SIG (46.3%) which similar to other reports. (3-5). The antimicrobial susceptibility test of 25 isolates of SIG to all antimicrobial agents show that all of isolates were resistant to at least one kind of antimicrobial agent. The isolated SIGs demonstrated the highest degree of resistance to aztreonam (76.7%). Additionally, isolated SIGs resisted amoxycillin (68.7%), ampicillin (64%) enrofloxacin (24%) and ciprofloxacin (16%). Resistance of SIG to cephalosporins revealed a broad range from 13.3-76.7%. The results reveal that isolated SIG resists to several types of cephalosporins, whereas no reports of cephalosporins resistant SIG from other reports (3, 5).

References

1. Barrow, G. et al. 1993.
2. CLSI, 2007 antimicrobial susceptibility testing standards M2-A9 and M7-A7.
3. Ganiere, H. et al. 2005. J. Vet. Med. 52: 25-31.
4. Ihrke, P. 2001. Infectious Diseases of Dog and Cat.
5. Sasaki, T.K. et al. 2007. J. Clin. Microbiol. 45: 2770-2778.
6. Werckenthin, C. et al. 2001. Vet. Res. 32: 341-362.

P20 The Diagnosis of Streptococcal Toxic Shock Syndrome (STSS) in a Dog

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Keywords: *dog, polyarthritis, Streptococcal toxic shock syndrome, valvular endocarditis,*

Introduction and Objectives

Streptococci are a family of gram-positive bacteria some of which can cause either localized or systemic even life-threatening infections in humans and animals. In dogs, Miller and Prescott (1996) reported a severe systemic disease and shock associated with necrotizing fasciitis caused by *Streptococcus canis* infection. The aim of this report is to investigate the rapid clinical progression and systemic effects of STSS on pathological changes in a dog.

Material and Methods

A 10-year-old, male, Golden Retriever was submitted to the Small Animal Teaching Hospital, Faculty of Veterinary Science, CU due to weakness of both hind limbs. Radiography revealed moderate DJD of both hip joints. Eleven days later, this dog returned with lateral recumbency, subacute necrotic cellulitis at the right thoracic wall, fever, panting, ulcerative stomatitis, increased lung sound and oliguria. Hematological and serum biochemistry analyses showed anemia, thrombocytopenia, leukocytosis and azotemia. Three days later, the dog was dead. Necropsy, cytology and tissue sections (fixed in 10% buffered formalin) for histological studies were performed.

Results and Discussion

Gross lesions showed purulent polyarthritis, valvular endocarditis (figure 1), purulent and hemorrhagic myositis and diffuse gastric ulcers. Microscopic examinations revealed fibrinosuppurative and proliferative arthritis, fibrinosuppurative valvular endocarditis with the present of bacterial clumps (Fig. 2c), multifocal membranous glomerulonephritis and lymphoplasmacytic interstitial nephritis. Cerebrospinal fluid (CSF) cytology revealed pleocytosis mainly with neutrophils (Fig. 2d). α-hemolytic streptococci were isolated from CSF and synovial fluid.

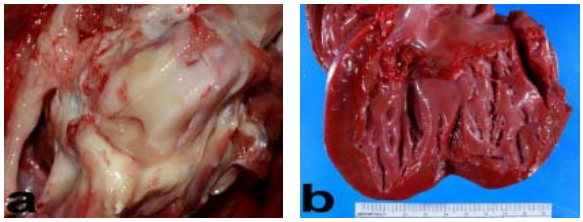

Figure1 Gross lesion; purulent arthritis (a) and valvular endocarditis (b)

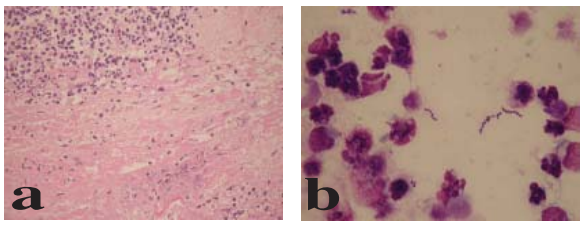

Figure 2 Histopathology and cytology; fibrinosuppurative valvular endocarditis (a) and neutrophils pleocytosis with presence of chain-like structure cocci (b)

The diagnosis is based on clinical history, pathological changes and bacterial culture. This dog showed rapid systemic symptoms including high fever, severe weakness and pulmonary edema leading to septic shock syndrome. Gross and microscopic lesions revealed generalized infection and culture of the affected sterile tissues yielded heavy growth of α-hemolytic streptococci. These results suggest that this dog died due to STSS.

References

1. Bert, F. and Lambert-Zechovsky, N. 1997. Clin. Microbiol. 35(3):777-779.
2. DeWinter, L.M. and Prescott, J.F. 1999. Can. J. Vet. Res. 63: 90-95.
3. Miller, C. et al. 1996. J. A.V. M. A. 209: 1421-1426.

P21 Identification of Coagulase-Positive Staphylococci causing Subclinical Mastitis and their Resistance to Penicillin and Oxacillin

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Keywords: *antibiotic, Coagulase-positive Staphylococci, mastitis*

Introduction and Objectives

Subclinical mastitis in lactating cows decreases both quantity and quality of milk. Coagulase positive staphylococci (CPS) are an important pathogen group causing bovine mastitis worldwide. Primiparous cows with CPS intramammary infection (IMI) at parturition may play a role of infectious source in dairy herds (1). Identification and antibiotic resistance information of this pathogen group have beneficial understanding of epidemiology and choice of antibiotics for treatment of mastitis.

Material and Methods

A total of 97 CPS isolates were collected from milking cows suffering from subclinical mastitis of during 2004 to 2008.

We identified CPS according to rabbit plasma coagulase tube test. API STAPH was used to identify CPS to species. Minimum inhibitory concentrations (MIC) of penicillin and oxacillin against CPS were determined with macrodiluion broth according to the method described by Clinical Laboratory Standards Institute (2).

Results and Discussion

The majority of CPS isolates were identified as *S. aureus* 91.75% (89/97). Others were *S. intermedius* 7.22% (7/97) and *S. hyicus* 1.03% (1/97).

Eighty nine of *S. aureus* isolates were classified into eight different API profiles. Fifty six isolates were grouped in the same API profile. Seven isolates of *S. intermedius* were classified into three different profiles.

MICs of penicillin and oxacillin against CPS tested isolates were between 0.0625 to > 1 μg/ml and 0.5 to > 8 μg/ml, respectively. The percentages of resistant Staphylococcus aureus to penicillin and to oxacillin were 65.17% (58/89) and 2.25% (2/89), respectively.

Other CPS, including *S. intermedius* and *S. hyicus* were all susceptible to both penicillin and oxacillin (Table 1).

Table 1. Penicillin and Oxacillin resistant CPS causing subclinical mastitis

	% Resistance	
	Penicillin[a]	**Oxacillin[b]**
All CPS	59.79 (58/97)	2.06 (2/97)
S. aureus	65.17(58/89)	2.25 (2/89)
CPS-non *S. aureus*[c]	0.0 (0/8)	0.0 (0/8)

[a]MIC ≥2.5 μg/ml: resistance

[b]MIC ≥4 μg/ml: resistance

[c]Use the same interpretive standard as *S. aurues*.

The majority of coagulase positive staphylococcal mastitis is *S. aureus*. Our study indicated that *S. intermedius* and *S. hyicus* were also able to be identified as CPS causing subclinical mastitis. However, both pathogens do not show any penicillin and oxacillin resistance. The predominant sources of *S. aureus* in dairy cow are IMI and heifer body sites (3). In the high prevalence *S. aureus* IMI herds, the majority of isolates belong to one or two strains (4).

The regular CPS sampling and antimicrobial resistance determination will provide the necessary information for choosing the most effective preventive measures for controlling mastitis.

References

1. Robinson, J.R. et al. 1994. J. Dairy Sci. 77: 958-969.
2. CLSI, 2007. Performance standard for antimicrobial susceptibility testing; seventeenth informational supplement. M100-S17 Vol. 27 No. 1.
3. Robinson, J.R. et al. 1994. J. Dairy Sci. 77: 3354-3364.
4. Tenhagen, B-A et al. 2007. J. Dairy Res. 74: 406-411.

P22 Cytochemistry of Blood Cells in Rice Field Frog, *Hoplobatrachus rugulosus* (Wiegmann, 1835)

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Introduction and Objectives

Rice field frog, (Hoplobatrachus rugulosus, Wiegmann, 1835), is a native frog in Thailand and has high economic importance (1). The blood cells of native Thai frog has not been yet reported (1). The purpose of this study was to demonstrate the cytochemical nature of blood cells of the rice field frog from Wang Nam Yen district, Sra-kaew province, Thailand.

Material and Methods

The anti-coagulated heparinized blood was obtained from the frogsí heart and subsequently made blood smear slides. These slides were air dried, fixed with absolute methanol and then stained with Wrightís Giemsa (WG). The cytochemical reactions of leukocytes were demonstrated using commercial kit (Sigma) for leukocyte alkaline phosphatase (LAP), Sudan black B (SBB), myeloperoxidase (MPO), acid phosphatase (AcP), non specific esterase (NSE) and periodic acid Schiff (PAS). The positive reaction was done in dog leukocyte samples which were pararelly stained (2).

Results and Discussion

The blood cells were identified based on their cytological organelles; nuclei and cytoplasmic granules similar to those of mammals (3, 4) (Fig 1-8). The morphologies of the erythrocytes and thrombocytes were similar to previous reported such as slender salamander (Batrachoceps attenuates) (4). Five types of leukocytes were classified as neutrophils, eosinophils, basophils, monocytes and lymphocytes and they were generally larger than mammals (4). The granulocytes were demonstrated obviously granules i.e eosinophil and basophil similar to other chelonian (2). The cytochemistry of leukocytes have been shown in Table 1. It is shown that neutrophils and monocytes showed their phagocytic activity in the inflammatory response as in mammals (4). The obtained data are useful for health monitoring of the natural frogs.

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Table 1 Cytochemical staining reactions of blood cells from 3 Rice field frog leukocytes

	Neu.	Eos.	Baso.	Lym.	Mono.
LAP	+	+	+	-	+
SBB	+	+	+	-	-
MPO	+/-	-	-	-	+/-
AcP	+/-	+/-	+/-	+	-
NSE	+	+	+	+	-
PAS	+	+	+	+	-

+: positive, -: negative, +/-:weak, Neu.: Neutrophil, Eos.: Eosinophil, Baso.: Basophil, Lym.: Lymphocyte, Mono.: Monocyte, SBB: Sudan black B, MPO: Myeloperoxidase, LAP: Leukocyte alkali phosphatase, AcP: Acid phosphatase, NSE: Non specific esterase, PAS: Periodic acid Schiff

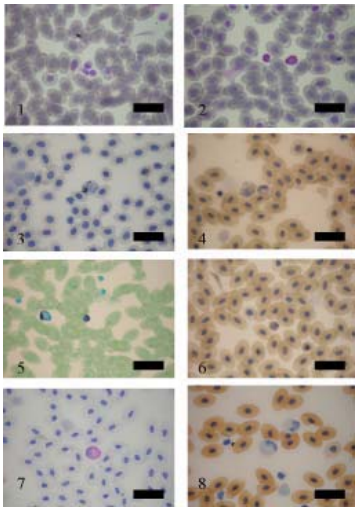

Figure 1-8 Photomicrographs of Rice field frog blood cells. 1. Neutrophil and lymphocyte, WG 2. Eosinophi demonstrated prominent reddish granules,WG 3. Neutrophil strongly positive to SBB 4. Neutrophil, LAP5. Lymphocyte, AcP 6. Neutrophill, NSE, 7. Eosinophil, PAS 8 Eosinophil negative to MPO. Bar = 20 μm

References

1. Chutmongkonkul, M. and Pariyanonth, P. 2007. 14th Proc. Assoc. Reptile and Amphi. Vet. USA: 118.
2. Assawawongkasem, N. et al. 2008. Proc. 7th Chula. Uni. Vet. Sci. Con.: 66
3. Raskin et al. 2000. Schalm's Vet. Hem. Lap.W&W, USA.: 37-345.
4. Thrall, M.A. et al. 2004. Vet. Hem. and Clin. Chem. Philadelphia: Lappincott W&W. USA.: 259-498.