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P1 THE STRUCTURAL STUDY OF THE SARTORIUS OF MEDIAL PATELLAR LUXATED DOGS

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Introduction and Objectives

Medial patellar luxation (MPL) is considered one of the most common abnormalities in small dogs². To develop corrective techniques, the surgeon must recognize the pathogenesis and the skeletal structures of the hindlimb. According to Nagaoka's study¹, it was found that postoperative physical exercise by flexion and extension of the stifle joint could promote medial strength, joint movement and stability. It was supposed that the sartorius muscles may pay an important role in the medial tensile force which caused the patella to luxate medially. This study compared the size and histopathology of the sartorius muscles of MPL, normal small sized and normal medium sized dogs. The results of our study will be considered for corrective technique selection.

Material and Methods

Sartorius muscles were studied in the MPL (group I) (20 stifle joints), and the small sized group (group II), as the control groups, and in the experimental dogs (group III) in each 10 stifle joints. Body weight and two hindlimb's circumferences (at the middle of the femur and at the proximal edge of the patella) were recorded. The sizes of the sartorius muscle were measured at the proximal edge of the patella (Fig. 1). The muscle samples were fixed and then processed by the histological techniques and stained with the H&E and Masson's trichrome, to evaluate the connective tissue and muscle fibers. The data was analyzed in order to evaluate the relationship between the sartorius muscle size, body weight and hindlimb's circumferences in the 3 groups.

Results and Discussion

The dogs in group II, who had normal patella, had a significant higher average size of cranial sartorius muscle than the dog in group I. It implied that dogs with MPL had a smaller cranial sartorius comparing it to the hindlimb's circumference ratio. Furthermore, dogs in group I had a lower ratio of cranial and caudal sartorius size and the hindlimb circumference than dogs in group III. The ratio of cranial sartorius size and hindlimb circumference at the mid femur area showed that the dogs in group I had lower ratio than groups II and III. Dogs with MPL had thinner and more tensile medial soft tissue than normal dogs which could pull the patellar out of the trochlear groove. Furthermore, the dogs with MPL had histopathological changes

in the muscle fiber such as hypertrophy, Zenker's degeneration, different muscle fiber sizes, interbedded collagen fiber, and a thickened muscle sheath. These changes may decrease muscle elasticity which may be the main cause or the outcome of MPL in small sized dogs (Fig. 2).

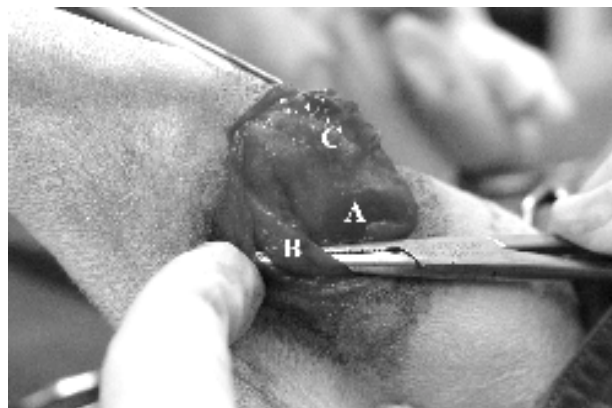


Figure 1 The sites of sample collection (A = cranial sartorius, B = caudal sartorius, C = patella)

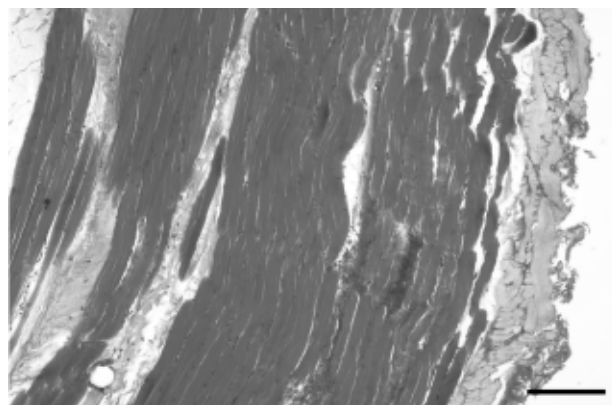


Figure 2 Histopathology of the striated muscle of the dogs in group I revealed the differences of fiber's size, dearrangement and replacement by connective tissue between the endomysium and perimysium. (Masson's trichrome stain, bar = 180 (μm)).

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P2 RADIOGRAPHIC POSITIONING OF THE CANINE ELBOW FOR IDENTIFYING THE MEDIAL CORONOID PROCESS

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Introduction

The definitive radiographic diagnosis of fragmented medial coronoid process (FMCP) is impossible because the fragment indicating the FMCP is always obscured by the superimposition of the radial head. A recent study showed that a disto 35 medial-proximolateral oblique (Di35M-PrLO) view, could enhance the identification of FMCP, compared to the conventional radiographic views¹. The objective of the present study is to find an alternative radiographic positioning of the elbow joint that aids the FMCP diagnosis.

Material and Methods

Elbow joints

Forelimbs of 20 euthanatized dogs free from orthopedic problems in or adjacent to the elbow joints, were included in this study. A one-millimeter diameter lead pellet was placed into the hole to represent the cranial margin of the subchondral bone of the MCP.

Radiographic positioning and acquisition

Radiography of the elbow joints using 3 paradigms of radiographic views, including craniocaudal, flexed 45 mediolateral, and extended 120 mediolateral, together with 3 different angles of distomedial-proximolateral (DiM-PrLO) oblique radiographic views, which are 25° (Di25M-PrLO), 35° (Di35M-PrLO), and 45° (Di45M-PrLO). Each DiM-PrLO was composed of 11 angled elbows, at 40°, 50°, 60°, 70°, 80°, 90°, 100°, 110°, 120°, 130°, and 140°.

Radiographic evaluation

All radiographs demonstrated the distance from the edge of lead pellet to the nearest radial surface by using a digital caliper (0.01 mm resolution).

Results

The results of the Di45M/PrLO with 40° flexed elbow revealed a significant minimum length between the pellet and the radial surface, compared to other oblique and conventional views ($P = 0.05$), as shown in Fig.1.

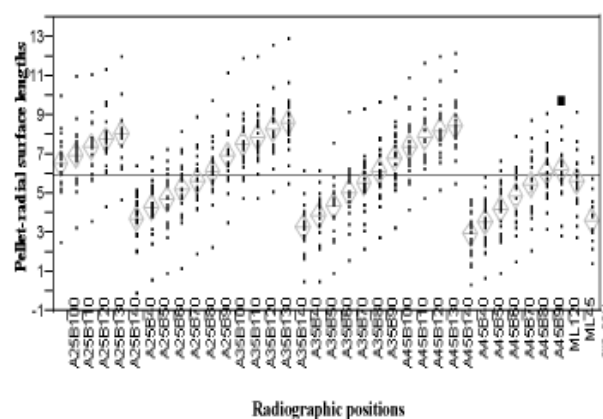


Figure 1 Means comparisons of the pellet-radial surface lengths between different radiographic positions. A = an angle of the proximal-distal elevation B = an angle of the flexed-extended elbow

Discussion

The present study indicated that the Di45M-PrLO with a 40° flexed elbow position enhances the identification of the cranial aspect of the MCP. As the primary fragmentation generally occurs on the cranial aspect of the MCP², this radiographic position can improve an assessment of the FMCP.

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P3 MICROVASCULARIZATION OF THE BRAIN IN GOLDEN HAMSTERS (*MESOCRICETUS AURATUS*)

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Introduction and Objectives

Nocturnal and burrow-dwelling animals with large eyes, large erect ears, and large cheek pouches, in which to store food, are golden hamsters (*Mesocricetus auratus*), with microvascularization of the brain as an important system to maintain normal life. Very little information concerning the microvascularization of the hamster's brain has been reported, so that, it is interesting to investigate and collect data, for comparison with other rodents and humans and applying it to vascular pathology in the future.

Material and Methods

Eight adult golden hamsters with a mean weight of 130 g were used and divided into two groups. The first group was used for macroscopic observation of the hamster brain. The second group was processed for study using a vascular cast technique. The brains from both of the brain groups were preserved in 10% formalin solution and were studied under a stereomicroscope.

Results and Discussion

It has been suggested that the major vascular channels at the base of the brain are through by way of the carotid and vertebrobasilar systems. They form a complete cerebral arterial circle (Figs. 1,2). The rostral and lateral part of this circle consists of an internal carotid artery (1) on the each side, and is divided into a rostral (5), middle cerebral (6) and caudal communicating artery (7). The rostral cerebral arteries are joined to each other by a single artery, the rostral communicating artery (4). Both of the caudal communicating arteries connect with the caudal cerebral artery (8) and form the caudal part of circle. The basilar artery (3) is formed by a merging the right and left vertebral artery (2) and the ventral spinal artery (13) joining at the midline. From caudal to rostral, the branches

originating from the basilar artery are as follows: caudal cerebellar (10), pontine (11), labyrinthine artery (12) divided terminally into the rostral cerebellar artery (9) and the caudal cerebral. These are characteristic of the circle in the golden hamster brain is the same as in the rat¹ and humans³, where according to guinea pig studies², the circle was formed from the internal ophthalmic, basilar and internal carotid arteries.

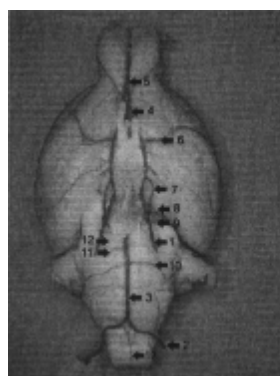


Figure 1 Stereomicrograph of golden hamster brain.

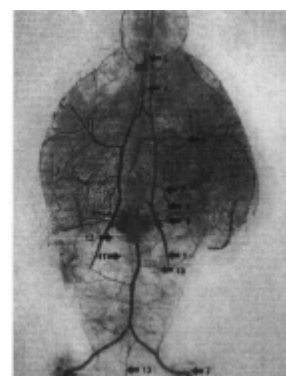


Figure 2 Stereomicrograph of vascular cast of golden hamster brain.

Acknowledgement

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P4 THE EXPRESSION OF OESTROGEN RECEPTOR ALPHA (ER α) IN A BITCH WITH PYOMETRA COMPARED WITH A NORMAL BITCH AT DIOESTRUS

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Introduction

Ovarian steroid hormones and their receptors play an important role in physiological as well as pathological changes in the uterus¹. For further understanding of the mechanism involved in pyometra, in relation to steroid hormone receptors in the bitch, the difference of oestrogen receptor alpha (ER α) expression between a normal and a pyometra bitch at dioestrus was investigated in the present study.

Material and Methods

Uterine samples were collected during dioestrus from normal bitches and bitches with pyometra. Immunohistochemistry was used to investigate the expression of ER α in different compartments of the uterus.

Results

The results showed that ER α staining scores were significantly lower in the epithelia (SE and GE) of pyometra cases compared to the normal group, as shown in Table 1

Table 1

Group	SE	GE	ST	M
Normal	4.25 $\pm$ 2.3 ^a	6.00 $\pm$ 1.1 ^a	0.80 $\pm$ 2.0	2.00 $\pm$ 3.16
Pyometra	0 $\pm$ 0 ^b	2.91 $\pm$ 12.33 ^b	.67 $\pm$ 1.63	2.16 $\pm$ 2.40

SE= Surface epithelium, GE =Glandular epithelium, ST = Stroma, M = Myometrium.

Mean ($\pm$ SD) within the same column followed by the different superscript letters are significantly different (P <0.05).

Discussion

The present study showed that the expression of ER α in the epithelia was strongly downregulated in pyometra cases when compared to normal uteri, during dioestrus. The mechanism of this downregulation may be due to prolonged levels of progesterone and/or the suppression of oestrogen, in order to enhance uterine infection². However, the difference of ER α localization in the stroma and myometrium between normal uteri and pyometra was not so obvious.

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P5 AN IMMUNOHISTOCHEMICAL STUDY OF THE PROTIEN BCL-2 IN THE EPITHELIUM OF THE PORCINE OVIDUCT DURING AN OESTROUS CYCLE

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Introduction and Objectives

The oviduct provides the environment for sperm transport, storage, capacitation, fertilization and the initial development of the early embryo¹. Previous studies have found that spermatozoa in the sperm reservoir (utero-tubal junction and caudal isthmus) maintained their fertilization ability by various mechanisms, before the time of ovulation². Therefore, the porcine oviduct would appear to be the source of Bcl-2, the protein that maintains viability and anti-apoptosis of cells³, during the estrous cycle.

The aim of the present study was to determine the localization of Bcl-2 protein in the different segments of the pre- and post-ovulation porcine oviduct.

Material and Methods

Oviducts of 20 multiparous crossbred sows (Swedish Yorkshire x Swedish Landrace) were collected post-mortem at pre- (n=10) or post-ovulation stages (n=10). Tissue samples from the utero-tubal junction (UTJ), isthmus and ampulla were removed, immersion-fixed in paraformaldehyde (1% in 0.15M PBS) and paraffin-embedded. Immunohistochemistry (streptavidin-peroxidase complex method) was done on 4 µm-thick sections, using a primary monoclonal mouse anti-Bcl-2 antibody. Negative controls were carried out by omitting the primary antibody and porcine lymph nodes were used for the positive controls. The intensity of immunostaining which appeared in the epithelium of each segment, was evaluated by semiquantitative methods.

Results and Discussions

The Bcl-2 pretein was clearly detected in intracytoplasm of the epithelial cells (mostly ciliated cells) of UTJ (Fig. 1), isthmus and the ampulla during the pre-ovulation period. In contrast, the expression of Bcl-2

was lower in the oviductal tissues during the post-ovulation period.

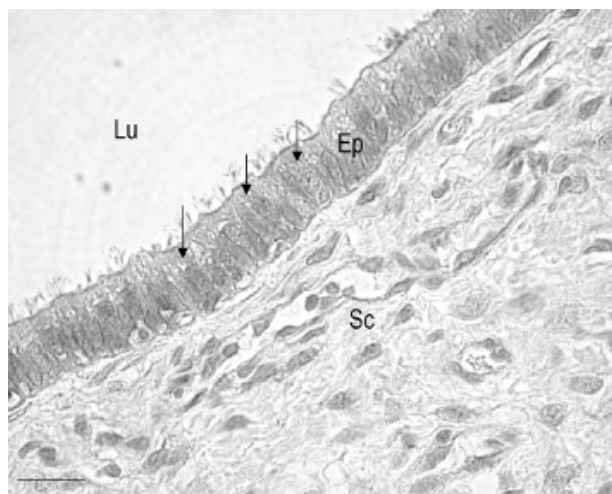


Figure 1 Immunostaining of Bcl-2 in the UTJ of the pre-ovulatory porcine oviduct, positive cells (arrows) are shown stained brown.

It has been found that Bcl-2 prevents apoptosis induced by a variety of stimuli and maintains cell survival by influencing the release of cytochrome c from the mitochondria³. Boar spermatozoa are often kept for 24-48 hrs in the UTJ before ovulation occurs. Thus, the expression of Bcl-2 in the pre-ovulation porcine oviduct, especially in the UTJ, could possibly play a role in an anti-apoptotic mechanism to protect spermatozoa during proestrus to estrus phases.

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P6 A STUDY ON THE NUMBER OF ER α AND PR POSITIVE CELLS IN THE MAMMARY GLANDS OF BITCHES DURING DIFFERENT STAGES OF THE OESTROUS CYCLE USING IMMUNOHISTOCHEMICAL ASSAY

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Introduction

The development of the mammary gland is influenced by numerous factors, principally oestrogen and progesterone that interplay with the action of various growth factors. The effects of oestrogen and progesterone are mediated via their respective receptors in the target tissues. Oestrogen and progesterone are essential for normal mammary gland growth and development. Oestrogen stimulates ductal growth during puberty, whereas progesterone is the major stimulator of mammary epithelial DNA synthesis and alveolar development². For further understanding of the mechanism involved in mammary gland tumors, in relation to steroid hormone receptors in the bitch, the expression of ER α and PR in normal mammary glands during different stages of the estrous cycle was investigated.

Material and Methods

Dogs were divided into 4 groups which were categorized by the stages of the oestrus cycle (proestrus, oestrus, dioestrus and anoestrus) using history data, vaginal cytology and serum progesterone. Twenty four samples of normal mammary tissues were obtained from 24 healthy, female dogs (1-5 years old) during the process of routine ovariohysterectomy (OVH). Incisional biopsy (approximately 1x1 cm of tissue sample) was performed. The investigation of ER α and PR was done by using Immunohistochemistry.

Result

Positive immunohistochemical staining was found in the nuclei of alveolar epithelial cells in the mammary tissues. A high intensity of ER α and PR-positive cells was found at proestrus and oestrus while a low intensity was observed at the other stages. The results showed that ER α and PR scores were significantly higher in proestrus and oestrus than other stages ($P < 0.01$), as shown in Table 1

Table 1

Stage	ER α score	PR score
Proestrus	28.45 $\pm$ 18.94 ^a	58.16 $\pm$ 24.59 ^a
Oestrus	25.30 $\pm$ 8.93 ^a	66.04 $\pm$ 9.95 ^a
Dioestrus	1.92 $\pm$ 2.99 ^b	16.08 $\pm$ 8.43 ^b
Anoestrus	0.32 $\pm$ 0.77 ^b	4.34 $\pm$ 7.03 ^c

Mean $\pm$ SD within the same column followed by the different superscript letters are significantly different ($P < 0.05$, N=24).

Discussion

The present study shows different levels of expression of ER α and PR in normal mammary tissue during various stages of the oestrous cycle. It seems that steroid receptor expression in mammary tissue is likely to be under the influence of steroid hormones, which are up-regulated by oestrogen and down-regulated by progesterone¹.

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P7 CLONED FLAT-HEADED CAT (*PRIONAILURUS PLANICEPS*) EMBRYOS PRODUCED BY INTERSPECIES SOMATIC CELL NUCLEAR TRANSFER

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Introduction and Objective

Flat-headed cats (FC), one of the small wild cats of Southeast Asia, are considered to be extremely endangered. Interspecies somatic cell nuclear transfer (iSCNT) is a beneficial tool, used for conservation of rare species, whose oocytes can not be obtained. The objective of the study was to investigate the development of the FC embryos produced by the iSCNT.

Materials and Methods

Immature oocytes of domestic cats were cultured for 24 h. The metaphase II plate of the matured oocytes was removed. A donor cell, a FC fibroblast, was transferred to enucleated oocyte. NT couplets were fused by inducing 3 DC pulses of 2.4 kV/cm for 50 μ s. Fused oocytes were activated and incubated in SOF, supplemented with 10% FCS, cycloheximide and cytochalasin B, for 4 h. Cloned FC embryos were cultured in SOF supplemented with 5% FCS, at 38.5°C, under 5% CO₂, for 7 days. Parthenogenic embryos served as controls.

Results and Discussion

The cloned FC embryos (Fig.1) reached the morula stage at a satisfactory success rate (Table 1), but there was blocking at the morula to blastocyst stage. However, the parthenogenic embryos in the study did develop to blastocysts with a great success rate. This indicates that the activation protocol used in the study is effective. Thus, the embryonic blocking may be caused by NT process, the ability of the donor cells and incomplete reprogramming¹ that should be clarified in further studies.

Conclusion

The study records the first success in producing cloned FC embryos using the iSCNT technique, and will be useful for producing offspring in the future.

Table 1. The developmental capacity of cloned FC and parthenogenic embryos.

Group	n	Fused	2-4 cell	Morula	Blastocyst
		n (%)			
FC	76	60 (79)	58 (97)	32 (53)	5 (8)
P	57	-	47 (82)	40 (70)	26 (46)

FC: flat-headed cat, P: parthenogenic embryo (4 replicates)

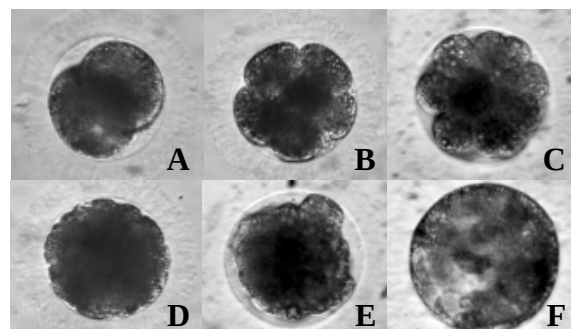


Figure 1 Cloned FC embryos at 2 cell (A), 8 cell (B), 16 cell (C), morula (D), compact morula (E) and blastocyst stages (F) (x 300).

Acknowledgements

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P8 THE DERIVATION OF MOUSE EMBRYONIC STEM CELL LINES FROM A HYBRID STRAIN

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Objective

The aim of this study was to establish germ-line competent ES cell lines from the hybrid mouse strain, B6D2F1.

Methods

The classical method^{1,2} to establish a mouse ES cell line was used with a slight modification. Without either the removal of the zona pellucida or immunosurgery, the 3.5 dpc blastocyst-stage embryos were placed directly into 24-multi-well dishes, precoated with mitomycin-C inactivated primary mouse embryonic fibroblasts (pMEF). The blastocyst embryos were cultured in Dulbecco modified Eagle medium (DMEM) high glucose, containing 2000 IU/ml leukemia inhibitory factor (LIF), 20% fetal bovine serum (FBS), 1x nonessential amino acids (NEAA), 0.2 mM 2-mercaptoethanol, 50 IU penicillin/ml, 50 mg streptomycin/ml, nucleoside and insuline transferine selenium (ITS) supplementation. The inner cell mass (ICM) outgrowths were picked mechanically with a fine pipette and disaggregated by gentle pipetting in 50 µl of 0.5% trypsin-EDTA. The disaggregated cells were replaced on the feeder cells until a stable cell line was established. The pluripotency of the established ES cell lines was determined by alkaline phosphatase staining, SSEA-1, Oct-4 immunostaining and chromosome number counting, after DAPI staining. In some cell lines, the embryoid body (EB) formation and spontaneous differentiation were also examined.

Results

Twelve ES cell lines were established from 36 blastocysts. The colonies had a smooth outline and were round or slightly oval in shape. All cell lines exhibited positive results for alkaline phosphates, SSEA-1 and Oct-4 staining. Cell lines number 3 and 4 exhibited the highest euploidy among the cell lines with a normal chromosome number.

Conclusion

The rate of hybrid ES cell line derivation achieved in this study, 33%, was similar to derivation rates of the widely available mouse strain, 129/SV. A further study will be undertaken to generate the germ line chimera of ES cell lines established in this study.

Acknowledgement

This work was financed by EU FP6 MEXT-CT-2003-509582, EU FP6 518240, Wellcome Trust - 070246, OTKA T046171 and the Thailand Research Fund (Royal Golden Jubilee Ph.D. Scholarship).

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P9 THE SUCCESS OF BOAR SEMEN CRYOPRESERVATION IN THAILAND: THE IMPACT OF STRAW VOLUME AND EQUEx-STM[®]

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Introduction

Frozen-thawed (FT) boar semen quality is affected by factors, such as the type of freezing package¹ and the detergent sodium dodecyl sulphate (SDS) in freezing extenders². The aims of the present study were to investigate the effect of straw volume (0.25 vs. 0.5 ml) and Equex-STM[®] (Nova Chemical Sales, Scituate Inc., MA, USA), a commercial preparation of SDS and added to a freezing extender, on boar sperm quality after cryopreservation.

Materials and Methods

Three ejaculates of semen from each of 4 boars (3 Landrace and 1 Yorkshire) were collected and frozen. Semen was diluted with isothermal Beltsville thawing solution (BTS) extender, held at 15°C for 2 h and centrifuged at 800xg for 10 min. The semen precipitant was re-suspended (1:1) with lactose-egg yolk (LEY) extender (80 ml of 11% lactose solution and 20 ml egg yolk). After further cooling to 5°C for 90 min, the semen were mixed with LEY extender and 9% glycerol, with or without 1.5% Equex-STM[®] (2:1). In experiment I, the processed semen (with 1.5% Equex-STM[®]) was loaded into 0.25 ml and 0.5 ml straws. In experiment II, the semen containing Equex-STM[®] and the Equex-free semen were only packaged in 0.5 ml straws. Semen-filled straws were placed in liquid nitrogen (LN₂) vapor for 20 min and plunged into LN₂ until being thawed. Thawing was achieved by immersing the straws in water, at 50°C, for 12 sec. The thawed semen was diluted (1:4) with an extender consisting of 95% BTS and 5% LEY extender

and incubated at 38°C for 30 min before evaluating the sperm quality. The data on sperm quality were analyzed using general linear model procedure (GLM) and SAS.

Results and Discussion

In the present study, the week of collection did not influence FT sperm quality. Boars significantly affected ($P<0.05$) FT sperm quality. It was revealed that FT individual motility (23.8% vs. 25.8%), and sHOST (10.5 vs. 11.5%) between 0.5 and 0.25 ml straws did not differ significantly, whereas the percentage of viable (24.0 vs. 31.4%) and normal acrosome (15.8 vs. 28.5%) spermatozoa were favorable for the 0.5 ml straws ($P<0.05$). This might be due to the freezing and/or thawing protocols used in this study which were inappropriate for 0.25 ml straws. The addition of Equex-STM, detergent, to the freezing extender improved ($P<0.001$) boar sperm membrane integrity (7.8 vs. 31.4%), motility (6.3 vs. 25.8%), and normal acrosomes (3.8 vs. 28.5%) after cryopreservation³.

Acknowledgement:

This study was funded by the Research and Development Center for Livestock Production Technology, Chulalongkorn University.

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P10 PIGLETS BORN AFTER INTRA-UTERINE ARTIFICIAL INSEMINATION USING FROZEN-THAWED BOAR SEMEN IN THAILAND

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Introduction

Frozen-thawed (FT) semen for pigs was developed many years ago³. However, its use under field conditions has been limited due to low fertility rates and impractical methodology. Recently, an intra-uterine artificial insemination (AI) techniques for pigs was developed in order to reduce the number of spermatozoa needed per insemination dose and increase the conception rates of FT semen². The aim of the present study was to assess the fertility results from FT boar semen, produced in Thailand, after intra-uterine AI in sows.

Materials and methods

Three purebred-boars (1 Landrace, 1 Duroc and 1 Yorkshire) were used as the semen donors. Collected semen was processed and frozen using a lactose-egg, yolk-based extender with 3% glycerol and 1×10^9 spz/ml in 0.5 ml straws according to the method described by Westendorf³ with some modification. For the insemination process: 7 weaned sows, parities 3-4, were tested for a standing estrus twice a day, by a back pressure test together with boar contact. These sows were inseminated 24 h after the onset of estrus, by deep intra-uterine artificial insemination (DIUI), with 1×10^9 spz/dose (2 sows), and intra-uterine artificial insemination (IUI), with 2×10^9 spz/dose (5 sows). The sows were re-inseminated 12 h later. During the insemination period, the ovulation times were monitored by transrectal ultrasonography. Pregnancy detection was performed by transcutaneous ultrasonography 28-30 d post service.

At farrowing, litter sizes, sex and the body weights of the piglets were recorded.

Results and Discussion

On 30 d post service, 5 out of 7 sows inseminated (2 DIUI and 3 IUI sows) were pregnant (71.4%). All pregnant sows farrowed normally. An inappropriate time of insemination, in relation to the ovulation time and early embryonic death, were suspected as the cause of pregnancy failure in 2 sows. The average total number of piglets born per litter was 9.4 (range 8-12), and comparable to the result of a previous study². The ratio of male:female piglets was 1.2:1, and the mean piglet weight was 1.1 kg. FT boar semen seemed to have no effect on either the sex or the body weight of the piglets. Nevertheless, it needs to be further investigated.

In conclusion, FT boar semen, produced in Thailand, can generate live offspring, at a satisfying level, after being used for deep insemination of sows.

Acknowledgement

This study was funded by the Research and Development Center for Livestock Production Technology, Chulalongkorn University.

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P11 IMPROVING THE REPRODUCTIVE PERFORMANCE OF SMALL HOLDER DAIRY FARMS USING A REPRODUCTIVE MANAGEMENT PROGRAM.

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Introduction

Problems in the dairy industry in Thailand are low milk yields and high production costs. A small dairy farm which has been neglected needs a proper management. Reproductive management is one of the first requirements for the farmers, who aim to reduce production costs, increase milk yields and calf production, while decreasing day-open of the cows.

The aim of this study was to monitor the reproductive performance of dairy cows in small holder farms after initiating a reproductive management program.

Materials and Methods

A total of 55 small holder farms (531 cows) from the Kampangsan dairy-cooperative, Nakhon Pathom province were used. The reproductive management program used in the current study was modified from a program used on a large scale dairy farm. Once or twice per month the reproductive organ were examined by rectal palpation and the cows were assessed into 4 groups; 1) those whose uterine involution occurred at 1 month post-partum 2) those that had normal uterine involution but were not inseminated within 2 months post-partum, 3) repeat-breeder cows and 4) those that were subjected to routine pregnancy diagnosis¹. After detecting reproductive problems, appropriate treatments were performed. The herd status and data were recorded and analyzed for reproductive efficiency.

Results and Discussion

To evaluate herd production efficiency, reproductive performance of the farm was compared with the target

number. In this study using a routine visiting program, the herd status of the farms (Fig. 1) was adjusted to a nearly ideal herd status³. In addition, the percentage of non-pregnant dry cows decreased from 3.7% to 0% within 10 months, while the percentage of pregnant-milking cows increased from 24.53% to 41.76% within 7 months.

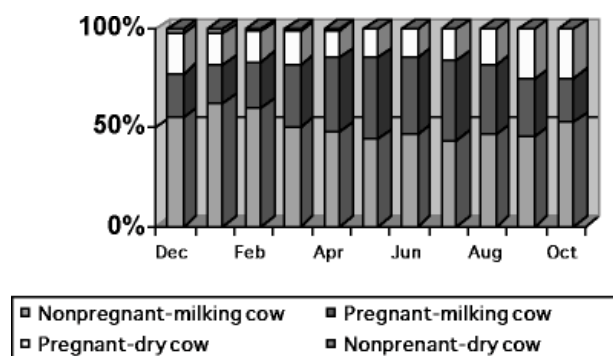


Figure 1 Herd status of the farm studied.

After 1 year, the conception rates at first service of heifers and cows were 66.7% and 52.6% compared to the target of 70% and 45%, respectively². In conclusion, an intensive management program facilitates the cow's reproductive performance in small holder dairy farms although acceptable costs of the veterinary services need to be considered.

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P12 SOW REPRODUCTIVE PERFORMANCE BEFORE AND AFTER IMPLEMENTATION OF AN ARTIFICIAL INSEMINATION SERVICE IN SMALL-HOLDER FARMS

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Introduction

Small-holder pig farms which hold less than 20 sows are owned by people in remote areas to generate extra money that increases the quality of life and allows saving. No boar is kept in those farms due to the high cost. A gilt or sow in oestrus is mated 2-3 times by a boar transported from neighbouring farm(s), who keep the boar(s) for rent. Artificial insemination (AI) had not been available in many provinces of Thailand. This study aims to implement AI services for small-holder pig farms and to investigate the reproductive performance after AI and the factors that influence it.

Materials and methods

A small scale boar station with an AI lab was setup. Two active boars with good genetics were used for semen collection. Boars were tested to be free from brucellosis, pseudorabies, and porcine reproductive and respiratory syndrome¹, they had no evidence of other clinical contagious diseases. Sow cards were used for reproductive data recording. A total of 171 sows from 86 farms, situated within a radius of 50 km, were included in this study. One hundred and twenty one sows were inseminated twice per oestrus by two inseminators who had been given standard AI training. Fifty sows were mated by rented boar. Sow reproductive performance from AI and boar services were compared. Housing, boar stimulation, population turn over rate and the distance

from the AI center to the farm were used as criteria for comparing sow reproductive performance.

Piglets per litter and weaning-to-oestrus intervals are shown as mean $\pm$ SD and were analysed using the GLM model. The non-return and farrowing rates are presented as a percentage and were analysed using generalized linear mixed models with Binomial errors. $P<0.05$ was set as the level of significance.

Results & Discussion

AI gave a better non-return and farrowing rate when compared with natural service (82%,74% vs. 74%,66% respectively). Platform housing, iron crate and boar stimulation all had a positive effect for AI results when compare to the effects of ground floor housing, wooden crate and no boar stimulation. (86.00% vs. 77.46%, 85.71% vs. 74.50% and 83.72% VS. 69.24% respectively). Boar stimulation improved litter size in this study (10.81 ± 2.45 vs. 9.01 ± 1.04 , respectively)². The results indicated that AI in small-holder farms had the same reproductive performance as natural service.

In conclusion, the type of housing and the presence of a boar during AI influenced the farrowing rate and the litter size when using AI service in small-holder farms.

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P13 THE INCIDENCE AND DURATION OF SUBCLINICAL MASTITIS IN HIGH AND LOW BULK MILK SOMATIC CELL COUNT SMALL DAIRY HOLDER FARMS

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Introduction and Objectives

Subclinical mastitis remains one of the most important problems in dairy herds which directly affects both the quantity and quality of milk. The California mastitis test (CMT) is a cow side test used for subclinical mastitis screening in herds. This observational study aims to determine the incidence and duration of subclinical bovine mastitis in high and low bulk milk, somatic cell count, dairy herds using weekly CMT for screening.

Materials and Methods

Forty small dairy holder farms were divided into two herd groups, according to their previous 3 month-geometric mean, bulk milk somatic cell count, either higher than 400,000 cells per ml (case group = 626 quarters) or lower than or equal to 400,000 cells per ml (control group = 752 quarters). All the quarters of the milking cows were tested for subclinical mastitis using the CMT once a week for twelve consecutive weeks.

Results and Discussion

Subclinical mastitis occurred in both groups. There were 137 and 134 positive quarters from 331 and 428 positive results during the 12 weeks in case and the control groups, respectively. (Table 1). The completeness of the CMT screening in case and control herds was 83.3 and 90.79%, respectively. The missing data was due to inconsistency of the CMT in the case group was higher than the control groups, which may have caused an incorrect estimation of the incidence and misdiagnosis as short duration subclinical mastitis. The duration characteristics were similar in both groups (only complete cases were included in both groups; Table 2.1-2.2). Subclinical mastitis mostly occurred one time only during the 12 weeks. It seemed to have a short duration in both groups (≤ 1 week). De Haas¹ exhibited that environmental mastitis pathogens can cause both short duration or acute cases.

Table 1 An estimation of subclinical mastitis in the case and control herd groups.

Group	Total cows	CMT	
		Number of positive quarters	Number of positive results during 12 wks
Case	159	137 / 626	331 / 1644*
Control	188	134 / 752	428 / 1608*

* $p < 0.05$

Table 2.1 The characteristics of subclinical mastitis duration in the case herd groups.

# Incidence	duration (week)		
	≤ 1	2	≤ 3
1	61	1	3
2*	17	3	3
3*	11	-	-

* Two or more incidences, duration were an average (week).

Table 2.2 The characteristics of subclinical mastitis duration in the control herd groups.

# Incidence	duration (week)		
	≤ 1	2	≤ 3
1	62	6	8
2*	16	3	9
3*	10	2	-

* Two or more incidences, duration given as an average (week).

The prevalence of subclinical mastitis can be controlled by decreasing the number of new cases or decreasing duration. The association between the type of pathogens in the herd, and the incidence and duration of subclinical mastitis, need to be further investigated.

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P14 THE EFFECT OF FEEDING DIFFERENT LEVELS OF CONCENTRATE ON THE FEED INTAKE AND FEEDING BEHAVIOR OF CROSSBRED PREGNANT HEIFERS.

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Introduction

Dairy cattle feed intake is found to be lower in pregnant than non pregnant cattle. This can reduce body weight and some animals will experience a negative energy balance before parturition. Feeding palatable feeds such as concentrate may overcome this problem. However giving too much concentrate can reduce roughage intake (RI) and result in lower chewing activity. This study was performed to study the effect of various level of concentrate on the total feed intake and feeding behavior of crossbred pregnant heifers

Materials and Methods

Four crossbred pregnant Friesian heifers, averaging 195±10 days into pregnancy, were randomly allocated to a treatment, in 4x4 Latin square design, which consisted of a 7 day preexperimental period and a 7 day collecting period. Treatments used 4 levels of concentrate, 0%, 0.42%, 0.87% and 1.29% of bodyweight, with corn stalk fed ad libitum (10% refusal) as a basal diet. Corn stalk was chopped to around 1-2 inches in length before being fed 4 times a day and concentrate, in form a powder, was given twice a day at 10.00 h and 14.00 h. Water was available at all time. Dry matter intakes (DMI) of both feeds were measured daily throughout the experiment. The bodyweight of each animal was measured 3 times on days 0, 8 and 14. On the last day of the collecting period eating behavior was recorded, using a video camera, for a 24 hour period. Total eating time (TET), total ruminating time (TRT) and total chewing time (TCT) were individually observed. TET, TRT, TCT/kg DMI and TRT/kg of neutral detergent fiber intake (NDFI) were calculated. All data was analysed with ANOVA. Means were analysed for difference by a least square mean test.

Results and Discussion

The DMI and DMI/%BW of animals in T1 and T2 was significant lower ($p < 0.05$) than in the other two groups (Table 1). RI of the animals in the concentrate feeding group was found to be substituted by concentrate. RI/%BW was significantly decreased ($p < 0.05$) when concentrate was fed. No difference was found in the NDFI. All levels of

concentrate decreased TET, TRT and TCT/kgDMI. ($p < 0.05$) (Table 2). All groups of animals spent more time eating than milking. Friesian cows at the same level of

Table 1 The effects of different amount of concentrate on BW, DMI, DMI/%BW of crossbred pregnant heifers.

Variable	0%	0.42%	0.87%	1.29%	SEM
BW (kg)	361	387.9	368.9	366.8	19.7
DMI (kg/d)	5.96 ^a	6.51 ^b	7.29 ^c	8.24 ^d	0.65
DMI/%BW	1.61 ^a	1.69 ^a	1.98 ^b	2.23 ^c	0.02
RI (kg/d)	5.96 ^a	4.89 ^b	4.06 ^c	3.42 ^d	0.56
RI/%BW	1.61 ^a	1.28 ^b	1.10 ^c	0.93 ^d	0.03
NDFI (kg/d)	4.06	4.00	4.04	4.18	0.28
NDFI/%BW	1.11 ^a	1.04 ^a	1.10 ^b	1.14 ^b	0.01

Different superscripts on the same row differ ($p < 0.05$).

Table 2 The effects of different amount of feeding concentrate on the feeding behavior of crossbred pregnant heifers.

Variable	0%	0.42%	0.87%	1.29%	SEM
TET (min/d)	442.3 ^a	373.0 ^b	344.3 ^c	321.0 ^d	25.0
TRT (min/d)	484.3 ^a	459.8 ^{ab}	405.3 ^{bc}	368.3 ^b	28.6
TCT (min/d)	926.6 ^a	832.8 ^b	747.0 ^c	689.3 ^b	46.3
TET/min/kgDMI	74.7 ^a	49.4 ^b	44.4 ^b	37.9 ^b	4.6
TRT/min/kgDMI	81.9 ^a	61.8 ^b	53.8 ^{bc}	44.7 ^c	4.1
TCT/min/kgDMI	155.6 ^a	110.3 ^b	98.2 ^{bc}	82.6 ^c	7.9
TRT/kgNDFI	227.5 ^a	185.0 ^b	177.5 ^b	162.8 ^b	15.4

Different superscripts on the same row differ ($p < 0.05$).

NDFI which had been reported to be 234 to 300 minutes for cows consuming 4 to 6 kg of NDFI/d¹. TRT/kgNDFI in each group showed significant differences ($p < 0.05$). The difference in TRT/kgNDFI was the effect of the amount of RI, and not the amount of NDF consumed. The roughage in this study had larger particle size than concentrate and was responsible for longer chewing activity time. Chewing activity is the response of the animals associated with the physical effectiveness of the NDF fraction³, and the particle size of the roughage² rather than the amount of NDF.

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P15 THE EFFECTS OF ROSELLE (*HIBISCUS SABDARIFFA* LINN.) ON THE ABSORPTION AND EXCRETION OF CALCIUM, PHOSPHORUS AND IRON IN POST WEANING PIGS

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Introduction and Objectives

Acid plays an important role in pH adjustments in the gastrointestinal tract, increasing enzyme activity and acting as a chelating agent to enhance the absorption of cations¹. Roselle 100 g is made up of 23 g of organic acids⁴.

Due to its acid property, the chelating effects of roselle on the absorption and excretion of Ca, P and Fe were investigated.

Materials and Methods

Forty-eight weaned crossbred pigs, aged 5 weeks and weighting 7.6 ± 0.05 kg, were randomly divided into 3 groups with 4 replications in each group. Treatment groups were fed a basal diet with either roselle 8% or an acidifier (Fra[®] Acid Dry) 0.4%. All feeds were calculated to meet the same nutritional values. Celite 0.2% was mixed in to all feeds for 3 days prior to slaughtering. To determine digestibility, the ileal and faecal contents were collected when the pigs were 7 and 9 weeks old. Minerals in the ileal and faecal contents were determined using atomic absorption (Ca and Fe) and alkaline ammonium molybdophosphate (P).

Data were analyzed by ANOVA using SPSS. Least squares means are presented and considered statistically significant at $P < 0.05$.

Results and Discussion

Table 1 The digestibility of Fe and Ca at the age of 7 weeks (%).

Digest.	Control	Roselle	Acidifier
Fe	99.17±0.41	99.41±0.14	99.14±0.46
Ca	99.91±0.03	99.95±0.02	99.93±0.03

No significant difference was found in the ileal digestibility of Ca and Fe among the treatment groups at the age of 7 weeks (Table 1). The high digestibility of Ca and Fe was due to its high availability in the sources i.e., oyster shell for Ca and ferrous sulfate³. However, roselle showed a better tendency than the others. Unfortunately, P was not determined due to insufficient contents.

Table 2 The excretion of Fe, Ca and P at the age of 9 weeks (mg/g).

Minerals	Control	Roselle	Acidifier
Fe	1.71±0.21	1.54±0.21	1.57±0.3
Ca	29.71±2.15	31.37±6.42	24.52±0.54
P	7.43±1.18 ^a	3.73±1.29 ^b	6.67±1.88 ^{ab}

When measuring the mineral excretion in faeces (Table 2), the pigs fed roselle excreted less P than the control group ($P < 0.05$). The tendency of the result is supported by Kemme², who showed that supplementation with acid to piglets could increase Ca and P digestibility compared to the control group.

It can be concluded that roselle probably performed as a chelating agent. Further experiments, however, need to be conducted for confirmation prior to any implementation of the roselle additive.

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P16 THE CYTOTOXIC EFFECT OF POLYSACCHARIDE GEL FROM DURIAN RINDS ON BOVINE MAMMARY LEUKOCYTES

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Introduction and Objectives

Thailand is one of the primary cultivators of durian. The polysaccharide gel (PG) from durian rinds has a variety of pharmaceutical benefits, such as immuno-modulator, antibacterial agent, gel and film forming agent¹. Preparation of a biomedical agent using PG was an attractive alternative for bovine mastitis prevention, since chemical agents for livestock are restricted. This study was undertaken to investigate the effect of PG from durian rinds on bovine mammary leukocytes.

Material and Methods

Mammary leukocytes were collected from udder of non-lactating dairy cows. Udder secretion samples were centrifuged, the cell pellet was resuspended and the cell suspension was adjusted to 5×10^6 in PBS.

The leukocytes were treated with 0.5, 1 and 2.5% PG (w/v) for 30 min. at 37°C and washed with PBS. Firstly, the cytotoxicity was determined by using a XTT reduction assay². Briefly, Cells were incubated with 1 mg/ml XTT and 25 µM PMS. Incubation was continued for 4 hours, and the colorimetric determination of formazan product was spectrophotometrically measured at 450 nm. The percentage of cytotoxicity was calculated. Secondly, the viability was quantified using a flow cytometric procedure with propidium iodide (PI)³.

A scanning electron microscope (SEM) was used to examine the morphology of the macrophages.

Results and Discussion

The results indicated that the cytotoxicity of PG on bovine mammary leukocytes were dose-dependent (Table 1). High concentrations of 2.5% PG exhibited a marked cytotoxic effect on mammary leukocytes. The SEM images of macrophages after treatment with PG (Fig.1) gave a similar result. 2.5% PG treatment inhibited or

inactivated the macrophages, whereas 0.5, 1%PG treated cells remained active.

Table 1 The percent cytotoxicity of PG on bovine mammary leukocytes (n =10).

Groups	%Cytotoxicity	
	PI	XTT
	Mean ± S.E.	Mean ± S.E.
Control (D.W.)	6.28 ^b ± 1.18	4.87 ^d ± 0.56
0.5% PG	4.37 ^b ± 0.82	15.22 ^c ± 1.27
1% PG	11.42 ^b ± 2.77	25.35 ^b ± 1.34
2.5% PG	27.61 ^a ± 2.81	70.08 ^a ± 3.97

a, b, c, d significant difference between groups ($P < 0.05$)

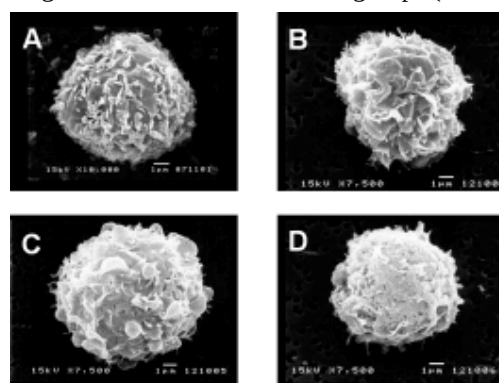


Figure 1 SEM diagram of treated macrophages with PG for 30 min. (x7,500). A) D.W. (control); B) 0.5%PG; C) 1%PG and D) 2.5%PG.

Further studies on bovine mastitis control and prevention using PG preparations need to be concerned about this cytotoxic effect.

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P17 THE EFFECTS OF *MORINDA CITRIFOLIA* L. (NONI) ON OXIDATIVE STRESS AND RENAL CATECHOLAMINE CONTENT IN DOXORUBICIN INDUCED NEPHROSIS IN RATS

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Introduction and Objectives

The mechanisms of doxorubicin (DOX) induced nephropathy are related to oxidative injury and renal sympathetic nerve activity. Tahitian noni juice (TNJ) shows inhibitory effects on lipid peroxidation with decreased central sympathetic outflow. The objectives of this study are to investigate whether TNJ prevents renal injury in DOX rats by modifying oxidative stress and renal sympathetic outflow.

Materials and Methods

The rats were divided into four groups. Group 1, control; group 2, TNJ; group 3, DOX and group 4, DOX + TNJ. TNJ was fed to group 2 and 4 per os daily for 28 days. Group 1 and 2 rats were injected with 0.9% saline solution while group 3 and 4 were injected with 7.5 mg/kg DOX i.p. on day 15. Rats were subjected to a renal function study on day 28. Both kidneys were removed to measure renal catecholamines and oxidative stress.

Results and Discussion

DOX caused a nephrotic syndrome¹. Increased plasma lipids were found. The glomerular filtration rate

decreased with increased blood urea nitrogen (Table 1). Sodium excretion decreased while protein excretion increased. TNJ reduced plasma cholesterol in normal rats and decreased Na⁺ excretion in DOX rats. Only renal dopamine (DA) and 3,4-dihydroxy-phenylacetic acid (DOPAC) were higher after DOX. DA causes renal vasodilation and natriuresis². Noni had no effect on renal catecholamine contents. Both renal GSH and catalase activity increased after DOX. TNJ caused a lesser increase in GSH and catalase activity, suggesting less oxidative damage³. It was concluded that rats receiving DOX had impaired renal function. This was compensated for by an increased DA and enhanced antioxidative process. Although TNJ modified oxidative stress, it showed no beneficial renal protection effects.

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Table 1 Effects of noni and DOX on renal functions, catecholamines and oxidative stress in 4 groups of rats.

	Day	Group 1	Group 2	Group 3	Group 4
BUN (mg/dl)	28	21.20 ± 1.01 ^a	19.90 ± 0.87 ^a	49.97 ± 6.23 ^b	39.16 ± 3.98 ^b
UNaV (uEq/day)	28	776.9 ± 109.0 ^a	551.2 ± 43.5 ^a	295.6 ± 58.5 ^b	202.7 ± 28.5 ^b
DA (pmol/mg prot.)	28	0.198 ± 0.021 ^A	0.260 ± 0.041 ^{AB}	0.363 ± 0.040 ^B	0.324 ± 0.053 ^{AB}
DOPAC (pmol/mg prot.)	28	0.268 ± 0.050 ^A	0.202 ± 0.016 ^A	0.437 ± 0.062 ^B	0.399 ± 0.033 ^B
Kidney GSH (nmol/mg prot)	28	33.54 ± 7.40 ^A	43.16 ± 8.58 ^A	138.26 ± 14.14 ^B	91.01 ± 12.54 ^C
Kidney CAT (unit/mg prot)	28	241.6 ± 42.4 ^A	280.3 ± 33.8 ^{AB}	374.5 ± 17.74 ^B	310.1 ± 17.3 ^{AB}

Data are presented in X ± SEM; Different superscripts indicate significant differences with P<0.05.

A,B,C and a,b compared data in the same row using one way ANOVA and Kruskal -Wallis on rank , respectively.

P18 THE EFFECTS OF BARAKOL EXTRACTED FROM CASSIA SIAMEA IN REDUCING THE COLONIZATION OF SALMONELLA IN YOUNG BROILER CHICKS

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Introduction and Objectives

Salmonella, especially *Salmonella* Enteritidis (SE), which predominantly colonizes gastrointestinal tissue of chickens, is the major cause of food-borne illness in humans. A reduction of salmonella contamination and colonization in poultry may help improve carcass quality as well as prevent human illness.

Barakol, a biologically active constituent extracted from *Cassia siamea*, has been shown to promote chloride secretion and to eliminate SE translocation in rat colonic epithelium^{1,2}. This study sought to determine the barakol action on the translocation and colonization of Salmonella in the gastrointestinal tract and visceral organs of young broiler chicks.

Material and Methods

Forty chicks (0 day old) were randomly divided into 4 groups: Gr1. control; Gr2. barakol; Gr3. SE and Gr4. barakol/SE. On days 1 and 2, chicks in Gr.2 and Gr.4 received barakol (50 mg/kg B.W) in the drinking water. 24 hours later, chicks in Gr.3 and Gr.4 were inoculated with 0.3 ml of 10⁹ CFU/ml of *S. Enteritidis*, serogroup D. The colonization of either natural (NS) or inoculated Salmonella in ileo-cecal tissues and spleen after 24 hours, 96 hours or on 11 day after inoculation, were isolated and counted.

Results and Discussion

The numbers of NS isolated from the ileo-cecal tissues of barakol-treated group were less than those of the control group 24 hrs and 96 hrs after treatment (Fig. 1A). In contrast, in the SE-inoculated group, the numbers of the inoculum SE strain, isolated from ileo-caecal tissues of control group, were significantly lower than those of the barakol treated group (Fig. 1B).

In addition, the NS were not found in the spleens of the barakol treated group but, in the control group, suggesting that barakol inhibits translocation of natural salmonella to visceral organs (Fig. 2A).

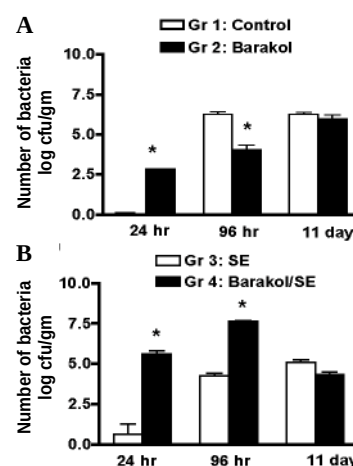


Figure 1 The histogram illustrates the number (mean±SEM) of SE isolated from the ileo-cecal tissues of (A) naturally salmonella-infected or (B) SE-inoculated chicken. *P<0.05 compared to the control or SE.

However, barakol treatment did not oppose inoculated SE translocation to the spleen

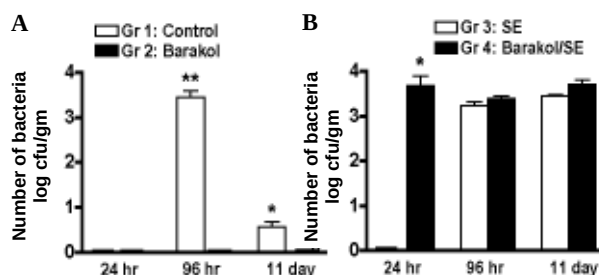


Figure 2 The histogram illustrates the number (mean±SEM) of SE isolated from the spleen of (A) naturally salmonella-infected or (B) SE-inoculated chicken. *P<0.05 and **P<0.01 compared to the control or SE.

The findings suggest that barakol may be benefit to improve the quality of carcass by decreasing the numbers of natural Salmonella contamination to meat.

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P19 THE ROLE OF ESTROGEN ON ANXIETY LEVELS AND NEUROTRANSMITTERS IN WISTAR RATS

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Introduction and Objectives

Anxiety is one of a group of the most common psychiatric problems that affect females more than males, especially following menopause. In different phases of the menstrual cycle, anxiety levels also vary, this also occurs in rats during the phases of the estrous cycle. In order to clinically treat anxiety, drugs that can modify the serotonergic system are the drugs of choice. Interestingly, women taking a selective serotonin reuptake inhibitor (SSRI), plus estrogen replacement therapy, showed fewer symptoms than those taking SSRI alone¹, suggesting a relationship between serotonin and estrogen on anxiety problems.

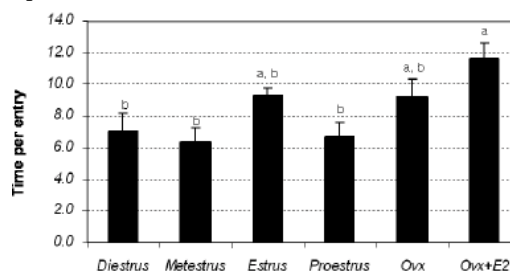
The present investigation was conducted to determine the role of estrogen on anxiety levels in female rats, using an elevated plus-maze (EPM), an accepted test for measuring anxiety in rats². Different ovarian hormone conditions (various phases of the estrous cycle, ovariectomized rats; Ovx and Ovx with estrogen supplementation; Ovx+E₂) were included. Additionally, the modulation effect of estrogen on brain serotonergic transmission was also analysed by measuring serotonin (5-HT) and its metabolite, 5-HIAA in different brain regions using high performance liquid chromatography with an electrochemical detector (HPLC-ECD).

Materials and Methods

Female Wistar rats were divided into 3 groups: Ovx, Ovx+E₂ (10 µg/kg, daily) and a sham control. The sham group was further divided into 4 groups according to the estrous cycle stage on the day of the behavioral test. Four weeks after ovariectomy, rats were tested with an EPM. After the EPM test, rats were sacrificed and the brains were immediately removed for 5-HT and 5-HIAA analysis, using HPLC-ECD.

Results and Discussion

Four weeks after ovariectomy, the body weight of the Ovx rats increased while the ratio of uterine weight to body weight and the serum estradiol decreased significantly, when compared to the others. This confirmed a deprivation of ovarian sex hormones in Ovx rats. The anxiolytic effect of estrogen was demonstrated by an increase in the time per entry in the EPM by the Ovx+E₂ group.



The serotonergic system was also affected by estrogen supplementation, which lowered the level of 5-HT and increased serotonergic activity in brain areas related to anxiety in the Ovx+E₂ group. However, during proestrous, associated with the highest level of estradiol, these showed a different effect from those supplemented with exogenous estrogen (Ovx+E₂); in that, they were more anxious than the Ovx+E₂ group. These results suggest that despite the high level of estradiol, the molecular mechanism of estrogen in normal cycling and chronic supplementation in controlling anxiety and/or serotonergic neurotransmission, was in fact different.

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P20 THE EXPRESSION OF THE AMINO ACID TRANSPORTER GENE SYSTEM L; LAT1, LAT2 AND 4F2hc IN THE MADIN-DARBY CANINE KIDNEY CELL LINE

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Introduction and Objective

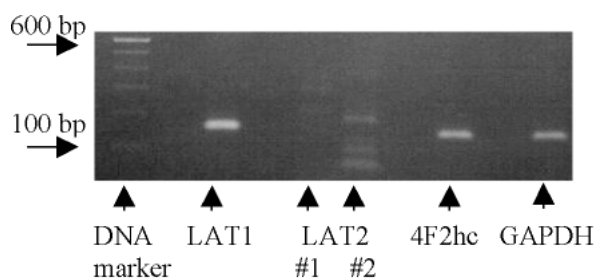
Among amino acid transport systems, the system L is considered to be one of the most important systems for sodium-independent neutral amino acid transport, since this system shows high activity for transport of both small and large zwitterionic amino acids and exhibits ubiquitous distribution in animal cells. The association of L-type amino acid transporter (LAT) subunits; LAT1 and LAT2, with a heavy chain of the 4F2 cell surface antigen, known as 4F2hc or CD98, plays an important role in the expression of transport activity at the cell surface. The Madin-Darby canine kidney (MDCK) cell, a well-differentiated renal epithelial cell line, is derived from a distal tubule/collecting duct. This monolayer cell may be useful model for evaluating transport of the absorptive amino acid, peptide, and monocarboxylic acid transporters. The expression of system L mRNA subunits and heterodimer can give useful information for studying the regulation of amino acid transport in relation to functional and pathophysiological characterization of dog kidney. The objective of this preliminary research was to investigate the mRNA expression of LAT1, LAT2 and 4F2hc in the MDCK cells because little information is available.

Material and Methods

Three sets of MDCK cells were cultured separately under standard conditions and using standard medium. To obtain the cDNA from MDCK cells, total RNA was extracted and first strand cDNA was synthesized. Primer pairs of each gene; LAT1, LAT2, 4F2hc, and GAPDH as an internal positive control, were designed and used for the polymerase chain reaction (PCR).

Results and Discussions

The results showed electrophoretic analysis of the PCR reaction revealed one specific band with the expected length of target amplified fragments of cDNA for LAT1, 4F2hc and GAPDH. On the other hand, PCR amplified product of cDNA for LAT2 showed non-specific bands, and this needed to be overcome by designing more specific primers for further investigation.



A group of renal tubular disorders; aminoacidurias may result in the loss of a single amino acid or a group of amino acids which are transported similarly and involve a defect in transport function, reflecting an abnormality in the transporter. For the clinical study of cystinuric dogs, a molecular biological approach should be done, because LAT2/4F2hc is a strong candidate gene for this investigation.

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P21 SPECIES IDENTIFICATION BY POLYMERASE CHAIN REACTION AND RESTRICTION FRAGMENT LENGTH POLYMORPHISM OF MITOCHONDRIAL DNA

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Introduction and Objectives

Mitochondrial DNA (mtDNA) will be the source material for species identification because the large numbers of copies of mtDNA present in each cell enhance the sensitivity of the analysis. One of the regions of mtDNA is a fragment of the gene encoding cytochrome b (*Cyb*).

We demonstrated a semi-nested PCR technique which increase sensitivity and used direct sequencing to authenticate the PCR products. Here we report the suitability of the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) techniques for animal and human identification purposes.

Material and Methods

0.5 g of bone powder from a cow, a pig, a chicken, a horse, an elephant and a dog or 100 µl peripheral blood mononuclear cells (PBMCs) from a cow, a buffalo, a goat, a sheep, a pig, a horse, a dog and a human were extracted by phenol/chloroform extraction. The DNA was amplified using semi-nested primers of the *Cyb* gene. The primary PCR mixture contained an upstream primer CYT b1; 5'-CCATCCAACATCTCAGCATGATGAAA-3' and a downstream primer CYT b3 ; 5'-TAGTTGTCTGGGTCTCCTAGGAGGTC3'. The secondary PCR mixture contained an upstream primer CYT b1 and a downstream primer CYT b2; 5'-GCCCTCAGAATGATATTGTCCTCA-3'. The DNA was subjected to cycle sequencing. We selected *Hae*III, *Hinf*I and *Mbo*I for species identification.

Results and Discussion

A fragment of the *Cyb* gene (359 base pairs) from all specimens was successfully amplified by semi-nested PCR. After digestion with *Hae*III, *Hinf*I and *Mbo*I and electrophoresis, each species yielded a characteristic pattern, as shown in Fig. 1. We confirmed the positive PCR result by direct sequencing. All these sequences were submitted to the GenBank database with accession numbers DQ236088-DQ236097.

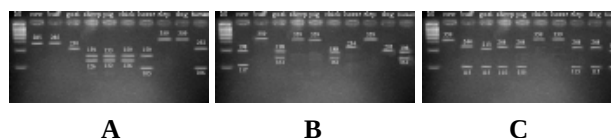


Figure 1 RFLP patterns of *Cyb* gene of a cow, a buffalo, a goat, a sheep, a pig, a chicken, a horse, an elephant, a dog and a human digested with endonuclease *Hae*III (A), *Hinf*I (B) and *Mbo*I (C).

M: 100-bp marker

In this study, we were able to differentiate between several species (cow, buffalo, goat, sheep, pig, chicken, horse, elephant, dog and human) using 3 restriction endonucleases (*Hae*III, *Hinf*I and *Mbo*I).

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P22 PHYLOGENETIC ANALYSIS OF A FELINE IMMUNODEFICIENCY VIRUS ISOLATED FROM A THAI CAT

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Introduction

Feline immunodeficiency virus (FIV) causes an immunodeficiency syndrome in cats. Sequences from the *env* gene from different isolates of FIV have been used to classify them into five phylogenetic subtypes A, B, C, D and E. Subtype A is found in the USA, Australia and Europe; subtype B in Japan; Europe subtype C in Canada, Europe and Taiwan; subtype D in Japan and Vietnam and subtype E in Argentina. This report is an attempt to investigate the subtypes of FIV isolates prevalent in Thailand.

Materials and Methods

A FIV, TH2, was isolated and identified from a cat with chronic urinary infection and admitted to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. Total RNA was extracted from saliva samples and the V3-V5 region of *env* gene fragments of the FIV were amplified by a reverse transcriptase polymerase chain reaction. The primer sequences were from those described by Pacino et al.⁴, as followed,

Outer primers

5'-GAGTAGATAC(A/T)TGGTT(G/A)CAAG-3' (nt 7134-7154)

5'-CATCCTAATTCTTGCATAGC-3' (nt 8145-8364)

Inner primers

5'-CAAAATGTGGATGGTGGAA(T/C)C-3' (nt 7326-7346)

5'-ACCATTCC(A/T)ATAGCAGT(G/A)GC-3' (nt 8165-8184)

The 859-bp-PCR products were purified and subjected for sequence and phylogenetic analysis and compared with those available in GeneBank database.

Results and Discussions

The 573-nucleotide sequence of the *env*-V3-V5 region of the TH2 FIV isolate was identified. The pairwise distances (% differences) compared to the same region in other FIVs were 20.64-22.01% from subtype A, 16.45-16.47% from subtype B, 19.70-21.25% from subtype C, 9.36-11.65% from subtype D and 17.32-18.07% from subtype E. The phylogenetic tree showed that the TH2 isolate clustered together with the FIVs in subtype D group (Fig. 1). Based on the partial *gag* sequence analysis, previously described, 6 Thai isolates belonged to subtype D¹. Subtype D is prevalent in Japan and Vietnam^{2,3}.



Figure 1 The phylogenetic relationship of nucleotide sequence in the V3-V5 region of *env* gene of FIV indicated that the Thai isolate, TH2, belonged to subtype D.

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P23 THE DETECTION OF GUPPY (*POECILIA RETICULATA*) MYCOBACTERIUM BY THE LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) TECHNIQUE

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Introduction and Objective

Mycobacteriosis is a localized infectious disease in guppy cause by *Mycobacterium* spp. Most of the moribund fish show undifferentiated signs of disease such as being off-feed, sluggish swimming and skin ulceration. The classical diagnosis of the disease has been done by histo-pathological demonstration of multifocal granulomas in the visceral organs of the fish and confirmed by bacterial isolation which needed much time to obtain results. LAMP is an emerging technology for the detection of microorganisms which become widely used. The advantage of LAMP is the rapidity of the reaction because there is no need to denature a double strand into a single strand DNA, as the reaction takes place under isothermal conditions. The technique is an autocycling strand displacement, DNA synthesis, performed using a DNA polymerase with a high level of strand displacement activity and a set of specially designed inner and outer primers. The objective of this study was to apply the LAMP technique for mycobacterium detection in guppy.

Materials and methods

Mycobacterium The mycobacterium, strain Thai 4, was isolated from guppy in a disease outbreak in Nakornpathom province. It was cultured and multiplied in Okawa-egg medium.

DNA template and primers A DNA template from Thai 4 was extracted using an isopant kit (Nippon Gene, Tokyo, Japan). Two inner (FIP: 5'-GAGCTGACGACA GCCATGCATTTTCGGCAGAGATGTCGGTTC-3', BIP: 5'-GTTGGGTAAAGTCCCGCAACGATTTTGGA GTCTCTCACGAGTCC-3') and outer (F3: 5'-GGGT TTGACATG CACAGGAC-3', B3: 5'-ACCTTCC TCCGAGTT GACC-3') primers were designed using Primer Explorer software (<http://www.venus.netlaboratory.com/partner/lamp/index.html>).

The LAMP reaction was carried out using a Loopamp kit (Eiken Chemical Co.Ltd., Tokyo, Japan). To optimize the amount of LAMP products, the reaction temp was compared at 60, 63 and 65°C while at 65°C the incubation time was compared over 15, 30, 45 and 60 min. The final acquired product was analyzed in 2.0 % agarose gel by electrophoresis.

Results and Discussion

The LAMP product was formed both at 63 and 65 °C but did not form at 60°C. No amplification of product was found with a reaction time of 15 min. However, at 30, 45 and 60 min amplification at 65°C, many bands of various sizes in the loading well were produced. This study is the first report of guppy mycobacterium identification using LAMP. Although there was limit number of bacterial strains in the study, the technique has shown the possibility of becoming a rapid diagnostic tool, which can be performed within 60 min.

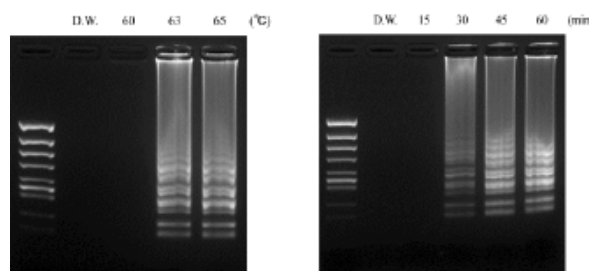


Figure 1 Effect of temperature and time on the amount of LAMP product.

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P24 COMPARISON OF SILVER BARB (*PANTIUS GONIONOTUS*) MILT QUALITY PRESERVED AT 25°C AND 4°C

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Introduction and Objective

Artificial insemination of endangered fish species is one of the best method for fish conservation. One of the problems with the method is that the eggs and sperm are not available at the same time. Therefore, a method to collect and preserve fish milt ready for use is needed. Milt collecting and preservation by chilling is a simple method that can be immediately performed when male fish are available before transport to the breeding center. The objective of this study was to apply cryopreservative extender to preserve fish milt, using chilling technique and silver barb' milt as the study model.

Materials and Methods

Milt The maximum volume of milt from 20 adult silver barbs with an average weight of 400 g, was collected by manipulation.

Extender One thousand ml of Hank's Balanced Salt Solution (HBSS) composed of NaCl 8.0 g, KCl 0.4 g, CaCl₂ 0.16 g, MgSO₄ 0.2 g, Na₂HPO₄ 0.06 g, KH₂PO₄ 0.06 g, NaHCO₃ 0.35 g and C₆H₁₂O₆ 1.0 g was prepared and with an adjusted osmolarity of 350 mOsm/kg and stored at 4°C until used.

Preservation and evaluation The quality of fresh milt was evaluated before being diluted in HBSS in the ratio 1:100. The diluted milt was divided into 2 portions of 30 tubes each, one stored at 25°C, another stored at 4°C. Sperm motility was stimulated by distilled water and evaluated under a microscope; the dead-alive spermatozoa were stained with aniline blue and observed every 3 hrs.

Results and Discussion

The results showed that by chilling, storage can extend the spermatozoa's life span for up to 83 hours and motile sperm could be observed until the 78th hour. Milt

stored at room temperature can be kept for 33 hours but motile sperm could only be observed for 24 hours. The method of milt chilling preservation at 4°C with HBSS, can effectively extend the life span of silver barb spermatozoa.

Table 1 Fresh silver barb milt quality.

Parameters	Average value
volume (ml)	1.04 (0.3-2.25)
density	+++
color	milky
pH	7.43 (7-7.5)
osmolarity (mOsm/kg)	285.15 (270-296)
concentration (x10 ⁷ /ml)	877.40 (561-1330)
motility (%)	99.00 (100-93)
sperm viability (%)	98.00 (100-90)



Figure 1 Alive (white) and dead (dark pink) silver barb spermatozoa (aniline blue stain).

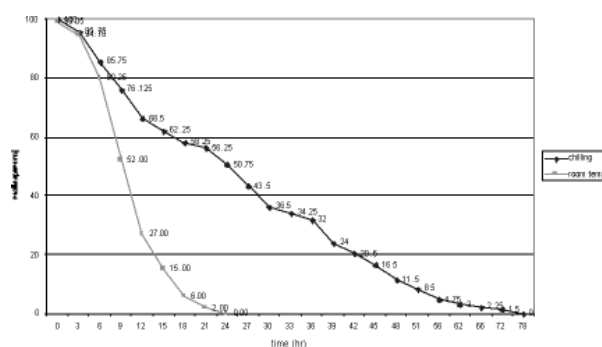


Figure 2 Comparison of sperm motility during 78 hours of preservation.

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P25 HISTOPATHOLOGICAL STUDIES ON ANOXIA AND HYPOXIA IN CATFISH

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Introduction and Objectives

Dissolved oxygen (DO) plays a potent influence on feed consumption, metabolic rate, energy expenditure and stress on fish¹. Anoxia and hypoxia conditions, with no and low DO in the water cause physiological responses. Histopathological studies of catfish tissues, when exposed to anoxia and hypoxic conditions have not been well documented.

This study was designed to investigate the effect of anoxia and hypoxia on pathological changes of catfish. The studied organs were gill, dendrite, kidney, liver, heart muscle, and body muscle.

Material and Methods

Four hundred catfish, 8.1 ± 2.1 g body weight, were divided into 2 groups. A long term (90 days) hypoxia group and a short term (3 hrs) anoxia group. Twenty catfish were put in each 300-L fiberglass tank. Each experiment was replicated five times.

Fish was euthanized and dissected after being subjected to anoxia and hypoxia. Six organs from each fish were fixed in 10% buffered formalin for histological studies.

Results and Discussions

In all catfish groups, there were no significant histopathological differences in gill, dendrite, kidney, liver, body muscle and heart muscle. It was noted only in some fish, that there were congestion in the gill lamellae (Fig.1) and increased mucous secretion in the dendrite epithelium cells (Fig.2).

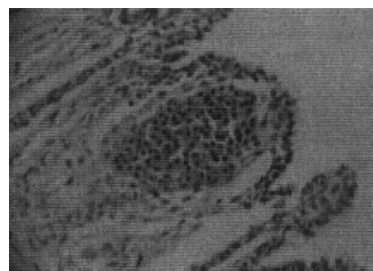


Figure 1 Blood congestion in the gill lamellae.



Figure 2 Mucous cell in the dendrite.

Catfish that survive after anoxia and hypoxia conditions show a physiological adaptation phenomenon².

In conclusions, the target organs of anoxia and hypoxia are the gill and the dendrite. Anoxia and hypoxia are not stressful for catfish.

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P26 THE ANTIMICROBIAL EFFECTS OF GUAVA LEAVE (*PSIDIUM GUAJAVA* LINN.) EXTRACT AGAINST *AEROMONAS* *HYDROPHILA* IN FANCY CARP (*CYPRINUS CARPIO*)

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Introduction and Objectives

Guava leaves have long been used as a herbal medicine in traditional medical practice. The antibacterial active ingredients in guava leaves are terpenoid, tannin and flavinoids which consist of Quercetin, Quercetin-3arabinoside and Guajavarin¹. The objective of this study was to determine the effects of guava leaves in the treatment and prevention of bacterial infection, using a methanol extraction of guava leaves.

Materials and Methods

Two hundred and four Fancy carps (*Cyprinus carpio*) with an average weight of 25.5 g. were acclimatized for 14 days before the experiment started. The fish were divided into 17 groups, 6 in each. All experiments were replicated. Dried Guava leaves were macerated and extraction was performed by methanol distillation and evaporation, to produce 12.99% of the dried leaf weight as the stock solution and kept at 4°C until use.

The treatments were 5% (MIC) and 10% (2xMIC) for the oral method, and 1000 ppm (MIC) and 2000 ppm (2xMIC) for the dip and bath methods. The minimum inhibitory concentration (MIC) of guava leaf extract against *A. hydrophila* was determined by an agar dilution method. Lethal dose (LD₅₀) of the guava leave extract, for carp, was determined to be 6×10^5 cfu intraperitoneally. Fish received guava leaf extract 7 and 14 days before and after challenge with *A. hydrophila* at LD₅₀.

The relative percent survival rate (RPS), and the Specific Growth Rate (SGR), the hematological value, blood chemistry, % phagocytosis, % chemotaxis and

water quality were measured. Statistical analysis was determined by using ANOVA (SPSS program) .

Result and discussion

MIC by the agar dilution method was at 1000 ppm. At 1000 ppm concentration and dipping for 5 minutes, the fish loss consciousness which was reversible when returned to freshwater. This may have been due to an antinociceptive effect². All fish died when given the 2000 ppm concentration dip. The RPS and SGR of the 5% feed mix group were significantly higher than the 10% feed mix group and higher again when fed for longer time. All groups that received guava leaf extract had a significantly higher % phagocytosis and % chemotaxis than the controls. (P<0.05). The results indicated that guava leaf extract can stimulate non-specific immune responses, increase the growth rate and decrease the mortality rate of bacterially infected carp. The effects were enhanced by a longer period of exposure and safer when given by the oral route

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P27 THE DETECTION OF CIRCULATING ANTI-GBM ANTIBODY LEVELS IN *EHRLICHIA SPP.* INFECTED DOGS

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Introduction and Objectives

Canine ehrlichiosis (CE) is an important disease in dogs worldwide, caused by rickettsia, Genus *Ehrlichia spp.*¹. The common clinical signs are fever, hemorrhage and renal involvement². The inflammation in the kidney can result from 3 major causes; immune complex crescentic glomerulonephritis, anti-glomerular basement membrane (anti-GMB) antibody and antineutrophil cytoplasmic antibody (ANCA). The objective of this study is to evaluate the relation between the anti-GMB and CE.

Materials and Methods

The samples were selected from 20 canine ehrlichiosis infected dogs, examined by giemsa straining of a buffy coat smear, and 20 healthy dogs from Bangkok. All infected dogs donated 5 ml of venous blood, 5 times, every 2 wks for CBC, Blood parasite, biochemistry and anti-GMB antibody evaluation. The anti-GMB was detected by IFA.

Results and Discussion

Clinical hematology of infected dogs was in the normal range although eosinophils in weeks 0, 2 and 4 increased. The platelet cell count was lower than the reference. Clinical biochemistry of infected dogs showed BUN, ALT and AST to be in the normal range. Creatinine in weeks 0, 2, 4 and 8 increased. ALP in weeks 4, 6 and 8 also increased (Table 1). All infected and normal sera were negative to anti-GMB antibody by the IFA. However, for clearer information, further experiments with induced disease in dogs should be carried out.

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Table 1. The clinical hematology and biochemistry of infected dogs.

Parameters	Units	Patient data (mean)					Reference range
		Wk 0	Wk 2	Wk 4	Wk 6	Wk 8	
RBC	x10 ⁶ /μl	5.8	6.1	6.2	6.4	6.5	5.5-8.5
Hemoglobin	g/dl	13.3	13.7	13.6	13.8	13.7	12.0-18.0
PCV	%	40.4	41.0	41.8	43.0	43.2	37.0-55.0
MCV	fl	62.9	61.4	61.4	61.1	61.2	60.0-77.0
MCH	Pg	23.2	22.8	22.2	21.7	21.1	19.5-24.5
MCHC	%	36.9	35.1	36.1	35.6	34.4	32.0-36.0
WBC	x10 ³ /μl	11.6	11.8	11.9	9.4	11.2	6.0-17.0
Neutrophil	x10 ³ /μl	5.8	6.2	7.2	5.7	6.7	3.0-11.5
Band	x10 ³ /μl	0.1	0.06	0.02	0.04	0.02	0-0.30
Lymphocyte	x10 ³ /μl	3.7	3.5	3.0	3.1	3.5	1.0-4.8
Monocyte	x10 ³ /μl	0.5	0.2	0.2	0.2	0.1	0.15-1.35
Eosinophil	x10 ³ /μl	1.4	1.8	1.3	0.4	0.7	0.10-1.25
Basophil	x10 ³ /μl	0	0	0	0	0	Rare
Platelet	x10 ³ /μl	175	173	170	169	138	200-500
BUN	mg/dl	15.2	16.0	15.1	16.9	17.3	7-32
Creatinine	mg/dl	1.6	1.6	2.2	1.3	2.3	0.5-1.4
ALP	IU/L	78.4	75.2	94.8	110.0	111.4	0-90
ALT	IU/L	45.6	27.8	30.7	29.3	26.4	10-94
AST	IU/L	32.1	25.2	20.2	13.6	21.1	10-62

P28 THE DEVELOPMENT OF ANTIBODY DETECTION AGAINST *HELICOBACTER PYLORI* BY IMMUNOFLUORESCENT ANTIBODY ASSAY (IFA)

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Introduction and Objectives

The immunofluorescent Antibody Assay (IFA) method has been widely used as a screening method. This study aimed to develop detection of antibodies against *Helicobacter pylori* by an immunofluorescent antibody assay (IFA) as a non invasive screening method for *Helicobacter pylori* infection.

Materials and Methods

Twenty sera from healthy New Zealand white rabbits and 20 sera from rabbits immunized against *Helicobacter pylori*, 5 times, weekly, and subcutaneously¹ were use for IFA evaluation. After observation by a dark field microscopy (Fig. 1), *H. pylori* were coated onto slides and fixed with cold acetone for 15 min. After air drying, the coated slides were incubated with the rabbit sera for 50 min at room temp. The slides were then washed with PBS 5 times, 1 min each time. The slides were then incubated with a goat anti rabbit IgG antibody conjugated with FITC, for 50 min at room temp. After PBS washing 5 times, the slides were evaluated by fluorescent microscopy.

Results and Discussion

All 20 normal rabbit sera were negative with an IFA (titer lower than 1:2) and *Helicobacter* induced sera had various titers from 1:4 to 1:16 (Fig. 2). Immunodiagnosis of *H. pylori* infection can be compared with other noninvasive diagnostic methods for the investigation of gastrointestinal symptoms. There are a number of different techniques for antibody detection². The development of antibody detection using the Immunofluorescent technique has now been performed in rabbits.

The results from this experiment could be useful to study the epidemiology and diagnosis of *H. pylori* infection.

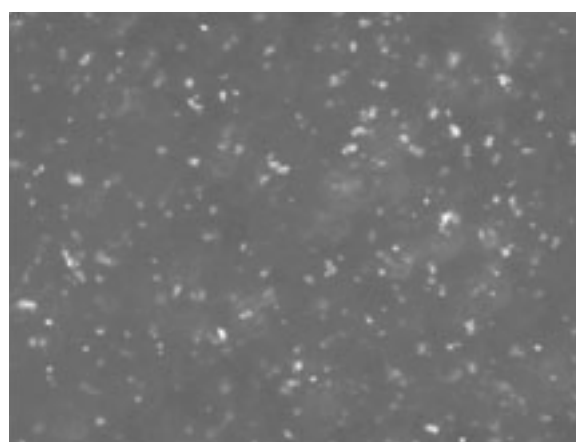


Figure 1 *H. pylori*, dark field microscopy x 400.

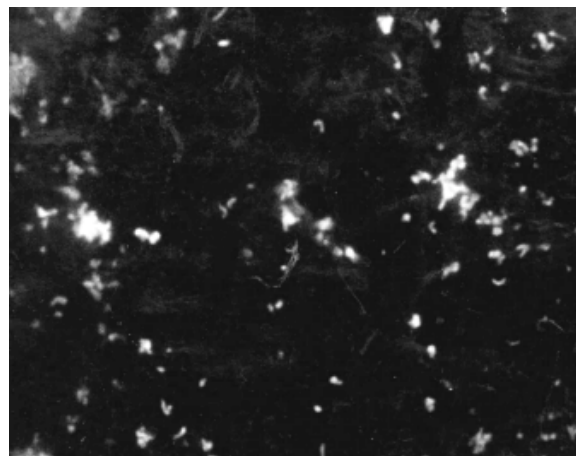


Figure 2 *H. pylori*, positive by indirect immunofluorescent assay (IFA) x 400.

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P29 THE EFFECT OF ONE- AND TWO-DOSE MYCOPLASMA HYOPNEUMONIAE VACCINE ON THE GROWTH PERFORMANCE OF PRRSV INFECTED NURSERY PIGS

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Introduction

Mycoplasma hyopneumoniae (MHYO) vaccination is an important strategy used to control mycoplasmal pneumonia¹. However, field observations suggest that vaccination in the presence of PRRSV resulted in increased disease severity. The objective of this study was to investigate the effect of MHYO vaccines on growth performance of PRRSV-infected nursery pigs.

Materials and Methods

The study was conducted in a farrow-to-finish farm, serologically positive for PRRSV and MHYO. Active PRRSV circulation was observed in the nursery unit in pigs 4 to 5 weeks of age. 160, 3-week-old pigs were randomly assigned into 8 treatment groups of 20 pigs each (Table 1). Each group was housed in one pen. Pigs were vaccinated with either one dose at 3 weeks old or two doses at 3 and 5 weeks old. Pigs were raised for 32 days. The average daily gain (ADG) and feed conversion ratio were compared between the groups. A clinical respiratory score² was performed on a daily basis. Sera were assayed for PRRSV specific antibodies by ELISA (IDEXX Laboratories, USA).

Results and Discussion

Pigs were PRRSV positive prior to nursery introduction, as measured by ELISA and PCR. Evaluation of nursery performance revealed a significant difference when using MHYO vaccines, in comparison with non-vaccinated controls, as the overall growth performance of vaccinated pigs was better than the non-vaccinated pigs (Table 1). Pigs vaccinated with the two-dose vaccine had a

lower clinical respiratory score and a higher ADG, compared to the non-vaccinated ones, and pigs vaccinated with oil-in-water, adjuvanted, one-dose vaccines. Pigs vaccinated with water-in-oil adjuvanted one-dose vaccines had higher ADG than other groups.

The results of the study suggest that the effect of MHYO vaccination on growth performance and health of PRRSV infected nursery pigs depends on the type of MHYO vaccines and the adjuvant. Two-dose products should be used to vaccinate pigs in the presence of PRRSV, as it induced a less negative effect on the pigs. One-dose products can also be used; however, an important consideration prior to use of one-dose products, to vaccinate PRRSV infected pigs, would be the type of adjuvant in the vaccine.

Table 1 Experimental design.

Gr	Types of adjuvant	Dose	ADG
1	NA	NA	399.22
2	Water in oil	1	421.09
3	Oil in water	1	371.56
4	Water in oil in water	1	420.78
5	Oil in water	1	385.94
6	AIOH	2	410.00
7	Oil in water/AIOH	2	418.56
8	AIOH	2	395.00

References

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P30 STUDY OF ANTIBODY RESPONSE TO LEPTOSPIROSIS VACCINE IN REPLACEMENT GILTS

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Introduction

Swine leptospirosis is a major zoonosis in pigs, caused by pathogenic *Leptospira interrogans*¹. Recently, many commercial combined leptospira vaccines have become available. The efficacy of such vaccine in an experimental laboratory show good antibody responses. However, the antibody responses in the field may give different results. Therefore, the objective of this study was to evaluate the antibody response of commercial combined leptospira vaccines in a real farm situation.

Materials and Methods

Thirty 6-7 months old, BW100-110 kg (LxR) gilts were divided into 3 group; Gr 1: 10 gilts which were vaccinated with Farrowsure gold B; Gr 2: 10 gilts which were vaccinated with Farrowsure B; Gr 3: 10 gilts which did not receive any vaccine. Gr 1 and 2 were vaccinated twice, on day 0 and 2 wk later. Sera from the pigs were collected 8 times; 2 wk before the first vaccination, the day of vaccination, 2, 4, 6, 8, 20, and 30 wk after the first vaccination. The antibody response was detected by the microscopic agglutination test (MAT) with 6 reciprocal leptospira serovars, bratislava, grippityphosa, pamona, hardjo, canicola and icterohemorrhagiae.

Results and Discussion

The MAT titer in Gr 1 against serovar bratislava, grippityphosa, canicola, and icterohemorrhagiae is shown in Fig. 1. The titer in response to serovar bratislava could be detected within 2 weeks of the first vaccination and reached a peak at the 4th week. The titer was sustained

until the 6th week and then the titer gradually decreased from the 8th to the 30th week after vaccination. The titers for the in other serovars (grippityphosa, canicola and icterohemorrhagiae) tended to be the same as bratislava. The MAT titer in Gr 2 is shown in Fig.2. The trend of the titers against other serovars was the same as Gr 1. However, the MAT titer against serovar grippityphosa in Gr 2 was lower than Gr 1. The MAT titer detected from other serovars, pamona and hardjo, were lower than 1:50. The MAT in Gr 3 was negative.

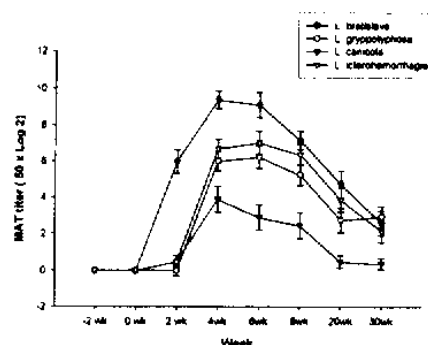


Figure 1 MAT titer in Gr 1.

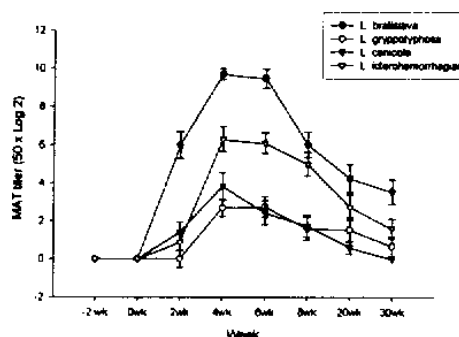


Figure 2 MAT titer in Gr 2.

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P31 AN EPIDEMIOLOGY SURVEY OF LEPTOSPIROSIS IN BREEDER PIGS FROM THE CENTRAL PART OF THAILAND USING A MICROSCOPIC AGGLUTINATION TEST

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Introduction and Objectives

Leptospirosis causes reproductive losses in sows¹. It is also a major pig-associated zoonosis worldwide. The gold standard for sero-surveillance is the microscopic agglutination test (MAT). In Thailand, leptospirosis is a major public health problem with regular outbreaks in humans annually³ and a high prevalence in rodent². However, studies on leptospirosis in pigs have been few. This study investigated the prevalence of leptospirosis in sows from central Thailand, which is the most important pig industrial region in the country.

Materials and Methods

One hundred and fifty pig sera (Gr.1 with 60 clinical leptospirosis sera, Gr.2 with 60 normal sera from the same farm with clinical leptospirosis and Gr.3 with 30 normal sera from a leptospirosis free farm) were obtained from farms in central Thailand from August 2004 to January 2005. All serum specimens were examined by MAT using 15 reference leptospira serovars, including australis, autumnalis, canicola, djasiman, patoc, grippityphosa, icterohaemorrhagiae, ballum, hebdomadis, bataviae, pomona, ranarum, shermani, tarassovi. Sera that gave a positive reaction at 1:50 dilution were further titrated, in a serial two fold dilution, to the titer end point, i.e. 50% agglutination.

Results and Discussion

The results showed that 46.6% of sows in Gr.1 were positive by MAT and the most prevalent was grippityphosa. The highest MAT titer was 50 (45%). In Gr.2, 18.3% of sows were positive and the most prevalent was grippityphosa. The highest MAT titer was 50 (10%) as showed in Table 1. Gr.3 had negative MAT. It is concluded

that leptospirosis is a problem in the pig industry of Thailand. Further investigation over a larger area should be carried out and disease elimination strategies should be performed.

Table1. Positive leptospirosis cases by MAT.

Group	MAT titer	Number of positive case for different leptospira serovars (%)		
		canicola	grippityphosa	patoc
Gr.1 (n=60)	50	1(1.7)	15(25)	11(18.3)
	100	0(0)	1(1.7)	0(0)
	200	0(0)	1(1.7)	0(0)
	400	0(0)	0(0)	0(0)
Gr.2 (n=60)	50	4(6.7)	0(0)	2(3.3)
	100	0(0)	2(3.3)	0(0)
	200	0(0)	1(1.7)	0(0)
	400	0(0)	2(3.3)	0(0)

The other 12 serovars were negative at the titer 1:50.

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P32 WEST NILE VIRUS TITERS IN *Aedes vexans* (MEIGEN) AND *Culex pipiens* (L.) SALIVA

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Introduction and Objectives

West Nile virus (WNV) is a positive, single-stranded RNA virus of the family *Flaviviridae*. It was originally isolated from a woman in the West Nile district of Uganda in 1937¹. This virus is present in Africa, the Middle East, Europe, Asia, and North America. Mosquitoes are involved in the transmission cycle of this virus in nature. WNV has been isolated from more than 30 species of mosquitoes. *Aedes vexans* (Meigen) and *Culex pipiens* (L.) are widespread mosquitoes. *Ae. vexans* predominately feed on humans and mammals. *Cx. pipiens* are bird feeding and WNV amplifying vectors.

This study was performed to illustrate WNV titers in infected *Ae. vexans* and *Cx. pipiens* saliva after ingesting different levels of WNV in blood meals.

Materials and Methods

Experimental animals. Day-old WNV neutralizing-antibody-free, broiler chickens were housed in biosafety level 3 facilities, at Iowa State University, USA.

Mosquitoes. *Ae. vexans* were the first generation, derived from adults collected in Iowa. *Cx. pipiens* were the 8th to 10th generations of mosquitoes originally collected in Iowa. They were allowed to feed on infected baby chicken with WNV titers ranging from 10^{4.8-8.8} PFU/ml of serum. Mosquito saliva was collected on day 14 post blood feeding by capillary feeding tube. Contents of individual capillary tubes were deposited into 600 µl of media following a 20-min feeding period. 300 µl of saliva solution was kept for virus isolation, and another 300 µl was kept for virus titration by plaque assay.

Virus. WNV (Iowa stain) was isolated from a brain of an infected crow that died in Iowa. It was propagated and assayed in Vero-76 cells.

Virus isolation. Virus in mosquito saliva was detected by virus isolation. Saliva solution was added into a cell culture flask containing a cell monolayer. Cell cultures were observed for CPE for up to 8 days and assays were confirmed by VecTest WN antigen assay.

Plaque Assay. Chick sera and mosquito saliva, positive for WNV by virus isolation, were tested by plaque assay using 6-well-plates.

Results and Discussion

Ae. vexans fed on blood meal with WNV 10^{5.1-8.8} PFU/ml and *Cx. pipiens* fed on blood meal with WNV 10^{4.8-8.8} PFU/ml. There were 94 *Ae. vexans* and 22 *Cx. pipiens* positive saliva when tested by virus isolation. The range of WNV titers in *Ae. vexans* and *Cx. pipiens* saliva were 10^{0-5.0} and 10^{0-4.7} PFU respectively.

This study indicated that WNV in *Ae. vexans* and *Cx. pipiens* saliva from a single mosquito was very high and might be high enough to infect humans and animals. These two species of mosquitoes might serve as important vectors in the WNV transmission cycle in nature.

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P33 THE EFFICACY OF THE COMPOUND, 10% (W/V) IMIDACLOPRID AND 50% (W/V) PERMETHRIN TO REPEL AND KILL *Aedes aegypti* MOSQUITOES ON DOGS

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Introduction and Objectives

Imidacloprid and permethrin are insecticides that have broad spectrum activity against insects and arachnids and low mammalian toxicity. The efficacy of the combination of these two insecticides has been examined with sand flies and ticks but it never tested with mosquitoes^{1,2}. *Aedes aegypti* is one of the most widespread floodwater mosquitoes. They are abundant, with widespread distribution and breeding potential in floodwater habitats. They readily feed on humans and animals so they can be a bridge vector for many pathogens between humans and animals. This study was conducted to assess the efficacy of an imidacloprid and permethrin combination to repel and kill adult *Aedes aegypti* mosquitoes on dogs.

Materials and Methods

Insecticide: A 10% (w/v) imidacloprid and 50% (w/v) permethrin preparation.

Dogs: Sixteen healthy dogs (local breed, either sex, over one year old).

Mosquitoes: A Liverpool strain of *Aedes aegypti* was maintained at $27 \pm 1^\circ\text{C}$ and $80 \pm 5\%$ RH and fed with a 10% sucrose solution.

Experimental Design: Two separate experiments were implemented each with eight dogs. Four dogs were in a treated group and the other four dogs were in a control group, for each experiment. 2.5 ml of the insecticide preparation was applied to each dog in the treated group and 2.5 ml of water was applied to each dog in the control group. Each dog was anesthetized and a hundred mosquitoes were allowed to feed on each dog, 10 and 3 days prior to

the insecticide application and 3, 7, 14, 21, and 28 days after the insecticide application.

Results and Discussion

There was no significant difference in the feeding success rates between the treated and the control group prior to the insecticide application. The feeding success rates of mosquitoes in the treated group were 4.9 and 4.4% on days 3 and 7 post treatment (p.t.), and increased to 6.3, 12.8, and 39.5% on days 14, 21, and 28 p.t., respectively. Feeding success rates between treated and control group on days 3, 7, 14, 21, and 28 p.t. showed significant differences. All mosquitoes in the treated group died on day 3 p.t. and mortality rates decreased to 97.1, 77.8, 40.4, 2.1% on days 7, 14, 21, and 28 p.t., respectively. Mortality rates between the treated and the control group after insecticide application showed significant differences, except on day 28 p.t. The percent efficacy of this insecticide combination were highest on day 3 and day 7 p.t. at 94.5% and decreased to 93, 86.5, and 58.5% on days 14, 21, and 28 p.t., respectively.

This study suggested that this insecticide combination can be used to repel and kill mosquitoes on dogs however the application might need to be repeated every 3-4 weeks.

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P34 A PATHOLOGICAL STUDY OF THE KIDNEY IN CATS INFECTED WITH FELINE INFECTIOUS PERITONITIS (EFFUSIVE FORM)

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Introduction and Objectives

Feline Infectious Peritonitis (FIP) is a fatal immune-mediated disease triggered by infection with a feline coronavirus (FCoV)¹. FCoV belongs to the family Coronaviridae, a group of enveloped, positive-stranded, RNA viruses that are frequently found in cats². FIP is a major factor in kitten mortality³. Difficulties in definitively diagnosing FIP arise from nonspecific clinical signs; lack of pathognomonic, hematologic, and biochemical abnormalities; and low sensitivity and specificity of the tests routinely used in practice. It was initially hypothesized that FCoV strains causing FIP are different from avirulent enteric FCoV strains⁴. It is now known that cats are infected with the primarily avirulent FCoV that replicates in enterocytes. A mutation occurs in a certain region of the FCoV genome, enabling the virus to replicate within macrophages, and causing the development of FIP. FIP (dry form or granulomatous form) has been known as one of the major causes of chronic renal failure in cats. Whether FIP (effusive form) cause any damage to the kidney in FIP-infected cats remain to be investigated. The objectives of this study were to study the pathological changes in the kidney of FIP-infected (effusive form) cats.

Materials and Methods

Kidney specimens from FIP-infected cats (effusive form) from the Pathology Unit, Faculty of Veterinary Science, Chulalongkorn University were studied from June 2002 to October 2003. Serum, effusive fluid protein analysis, and histopathology were used to confirm the diagnosis of FIP. Kidney specimens from FIP-infected

cats, stained with H&E and immunohistochemistry were used for pathological evaluation.

Results and Discussion

Histopathology diagnosis of kidney specimens of FIP-infected cats (effusive form) revealed tubulonephrosis 100% (5/5) and chronic interstitial nephritis 80% (4/5). Immunohistochemistry of kidney specimens revealed staining of the tubular epithelium, glomeruli and scattered in the interstitial area 20% (1/5). From the result of this study, it did not seem that FIP virus directly caused pathological changes in the kidney in FIP-infected (effusive form) cats.

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P35 A RETROSPECTIVE STUDY OF THE HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY OF PORCINE CIRCOVIRUS-2 INFECTION IN PORCINE LYMPHOID TISSUES AND LUNGS

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Introduction and Objectives

Porcine circovirus (PCV)-2, a small, single-stranded, circular DNA virus, is a cause of postweaning multisystemic wasting syndrome (PMWS) in pigs. Currently, the disease has been reported worldwide¹. In Thailand, we previously reported the detection of PCV-2 in weaning pigs²⁻⁴. The immuno-histochemical (IHC) detection of PCV-2 antigen from pig tissues has been reported to be sensitive, reliable and inexpensive. The purpose of this study was to examine the histopathology and the PCV-2 antigen in lymphoid tissues and lungs of pigs in Thailand, using the IHC method.

Materials and Methods

Formalin-fixed, paraffin-embedded (FFPE) tissue samples from 129 PMWS-suspected pigs (5-16 wks old), diagnosed during 2001-2003, were examined. The FFPE blocks were cut at 4- μ m-thick and stained with H&E and IHC methods. For the IHC study, a previously procedure (2) was used with polyclonal rabbit anti-PCV-2 antibody dilution 1:200, as the primary antibody and the avidin-biotin complex peroxidase method (ABC) with DAB visualization. The distribution and severity of the lesions were evaluated by semi-quantitative analysis.

Results and Discussion

The incidence of PCV-2 antigen detection increased during the years 2001-2003, by 30.3 % (10/33 cases), 40.48% (17/42 cases), and 42.59% (23/54 cases), respectively. Fifty out of 129 PCV-2-suspected cases (38.76%) were found IHC-positive to PCV-2 antigen. The percentage of positively stained tissues were found to be 40.70% (35/86) in the lymph nodes, 38.54% (37/96)

in the lungs, 36.99% (27/73) in the tonsils and 32.22% (29/90) in the ileum. Histopathologically, the average severity of lesions in the organs was 2.29(0.89 for lymph nodes, 2.16 $\pm$ 1.11 for ileum, 2.00 $\pm$ 1.16 for tonsils and 1.43 $\pm$ 0.55 for lungs. The average severity of PCV-2 antigen distribution in the organs was 2.28 $\pm$ 1.11 for lymph nodes, 2.23 $\pm$ 1.0 for ileum, 1.97 $\pm$ 1.77 for tonsils and 1.76 $\pm$ 1.28 for lungs. PCV-2 antigen was frequently found in the cytoplasm of macrophages. In addition, the PCV-2 antigen was also found in multinucleated giant cells, fibroblasts, lymphocytes and epithelial cells. This study revealed that the presence of PCV-2 antigen was primarily seen in the lymphoid follicles, with an extension to the paracortical sinus in severely affected pigs which was related to the severity of the histopathology. In the lungs, PCV-2 antigen was frequently found in interstitial macrophages that did not detect the inclusion bodies in the cells. The viral antigen was also occasionally detected in the bronchiolar epithelium, fibroblasts and lymphocytes. This study showed that lymph nodes appear to be the most PCV-2 antigen-positive organ, followed by the lung, tonsils and ileum.

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P36 THE PRESENCE OF SALMONELLA SPP. IN SMALL DAIRY HOLDER FARM

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Introduction and Objectives

Salmonella spp. infections cause both human and cattle diseases. There is an association between the *Salmonella* causing disease in humans and cattle. Non-typhoid *Salmonella* spp. are mostly isolated from human gastroenteritis. Up to date, there are nearly 2,500 salmonella serovars isolated from many hosts. Most bovine salmonella infections are subclinical but which animals excrete and cause the contamination of meat and dairy products.

Our aim was to determine the presence of *Salmonella* spp. in feces, slurry and bulk tank milk samples on small dairy farms.

Material and Methods

Slurry from small dairy farms in the central (n=273) and northern (n=37) regions of Thailand were collected for *Salmonella* spp. screening. In slurry salmonella-positive farms (n=11), we determined the presence of *Salmonella* spp. in feces and bulk tank milk samples. Isolation and identification of the *Salmonella* spp. were conducted according to the protocol described by ISO 6579 and serotyped by the WHO National Salmonella and Shigella Center of Thailand.

Results and Discussion

Salmonella spp. were isolated from 4% and 2.7% of the slurry in the central and northern region, respectively. Positive cultures for individual fecal samples were about 7.3% (central region) and 13.5% (northern region). The most common serovar among the 36 isolates from feces were *S. weltevreden* (54%) and *S. stanley* (19%). The most prevalent serovar isolated from slurry was *S. weltevreden* (37%). There was no *Salmonella* spp. in bulk tank milk samples. The result indicated that there is a risk of salmonella in dairy farms.

Table 1 Percent of salmonellar-positive cattle in small dairy farms in central and northern regions of Thailand.

Area	Percent of positive cattle				
	Calves	Yearlings	Heifers	Dry cows	Milking cows
Central region	13.6 (3/22)	4 (4/100)	0 (0/29)	7.9 (3/38)	10.8 (12/111)
Northern region	36.4 (4/11)	23.5 (4/17)	7.1 (2/28)	0 (0/8)	10 (4/40)

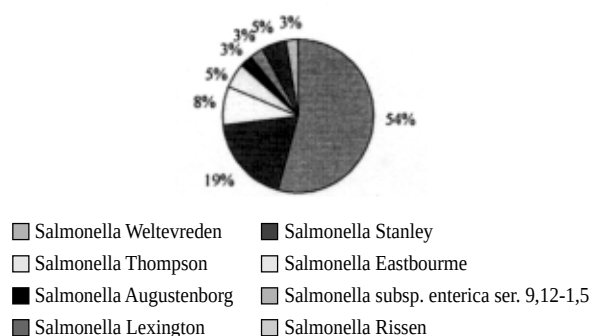


Figure 1 Salmonella serovars in feces

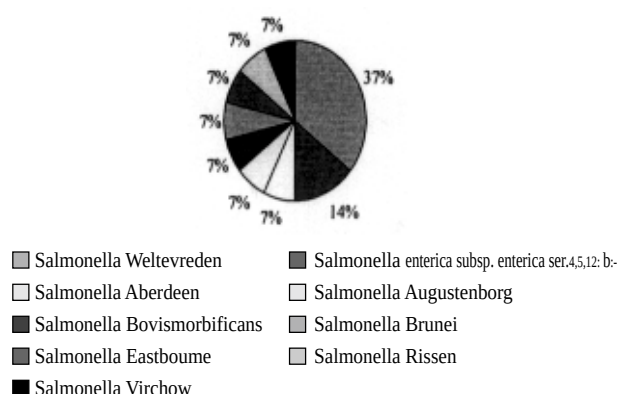


Figure 2 Salmonella serovars in slurry

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P37 THE SEROPREVALENCE AND IDENTIFICATION OF ORNITHOBACTERIUM RHINOTRACHEALE (ORT) FROM BROILER AND BROILER BREEDER FLOCKS IN THAILAND

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Introduction and Objectives

A major problem for the poultry industry around the world is a respiratory tract infection which can cause major economic impact. ORT is a slow growing, gram negative bacterium. ORT infections have been reported in many countries around the world. The objective of this study was to determine the prevalence of ORT infection, its isolation and identification in broilers and broiler breeders in Thailand.

Materials and Methods

Broiler and broiler breeder serum samples.

Serum samples were collected from 17 broiler farms and 23 broiler breeder farms from the major broiler producing companies of Thailand during the period Oct. 2004 to Sep. 2005. Samples were kept at -20°C until tested. **ELISA.** Sera were analyzed by indirect ELISA with an ORT antibody test kit. **Bacteria.** The bacteria were collected from chickens on the seropositive farms by swabbing from the air sacs of those with respiratory problems. The bacteria were cultured in a Columbia agar base with 5% sheep blood and 5 µg/ml gentamicin under microaerophilic condition, for 48-72 hr. Gram negative, catalase negative and oxidase positive colonies were tested by PCR. **PCR analysis.** Primers used in this study were OR16S-F1 (5'-GAGAATTAA TTTACGGATTAAG-3') and OR16 S-R1 (5'-TTCGCTTGGTCTCCGAA GAT-3'), which flanked a 784-bp DNA sequence. PCR was conducted. A reaction volume of 20 µl contained 10XPCR buffer with (NH₄)₂SO₄, 2.5 mM MgCl₂, 0.2 mM

dNTP, 1.0 µM of each primer, 50-100 ng DNA Template, 1.5U Taq DNA Polymerase. Samples were subjected to 94°C, 90 sec followed by 35 cycles at 58°C, 60 sec and 72°C, 90 sec. PCR products were examined in 1.8% agarose gels with 0.5X TBE buffer, stained with 10 µl of ethidium bromide (20 mg/ml), and exposed to ultraviolet light.

Results and Discussion

The broiler breeder ELISA results were 12.2%, 38.0%, and 49.8%, neg., suspected and pos., respectively. The broiler ELISA results were 67.5%, 12.9% and 19.6%, neg., suspected and pos., respectively. ORT vaccines have not been used in Thailand so, the suspected results may be regarded as positive results. The suspected+positive results in the broiler breeder farms are 87.84% and the older birds were more positive than the younger birds. The suspected+positive results in the broiler farms were 32.5%. Nine isolates of the twelve PCR analysis samples revealed positive results to PCR analysis. Our studies are the first report confirming seropositive birds and the incidence of ORT in Thailand.

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P38 A CARDBOARD BOX USED AS A PREVENTIVE MEASUREMENT FOR *EIMERIA* INFECTION IN CHICKEN

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Introduction

Avian coccidiosis is an intestinal disease caused by protozoan parasites, of the genus *Eimeria*. Coccidiosis is difficult to eliminate from the poultry rearing process and therefore commercial chickens free from *Eimeria* sp. are extremely rare¹. A technique to rear young chicken between one to ten days old was developed in our lab. Rearing chicken were transferred from a birdcage into a cardboard box. The box made from household material to replace the cage and is absolutely coccidiosis free. All birds were tested for *Eimeria* infection using the Polymerase chain reaction (PCR) technique. Rearing young chicken in this system is most convincing as a tool for laboratory use.

Materials and Methods

A cage: A large rectangular cardboard box was cut, refolded and provided with a wire mesh floor. A food trough and water dispenser were also built from a household recycled paper box and a plastic bottle (Fig. 1).

***Eimeria* sp.:** One day old chicken were inoculated with a 1 ml suspension of 100-10,000 *Eimeria* CB38 oocysts in distilled water. On day 4 after infection, chicken were sacrificed and using PCR were tested for *Eimeria* infection.

The PCR technique: This technique was previously described in detail². The 422 bp fragment is amplified from the 18S rRNA gene.

Results and Discussion

Chicken from each group were tested for the DNA of *Eimeria* in the intestine by PCR (Fig. 2). *Eimeria* was found in all parts of intestinal tract (10⁴ oocysts). Meanwhile, *Eimeria* in birds infected with 10² oocysts could not be detected on day 4 but later on day 5. The control group had no infection throughout the experiment (No oocysts). PCR amplification is highly sensitive and can easily detect the genetic material of pathogens in animal tissues. The results demonstrated that rearing chicken in a cardboard box is proven to be free from coccidiosis. Ones that were infected were also negative for other exogenous oocysts.



Figure 1 A cardboard box used as a chicken pen. This box is cut and refolded. Wire mesh is modified to be a cage floor. Food trough and water dispenser are attached. When use, this box is covered with a piece.

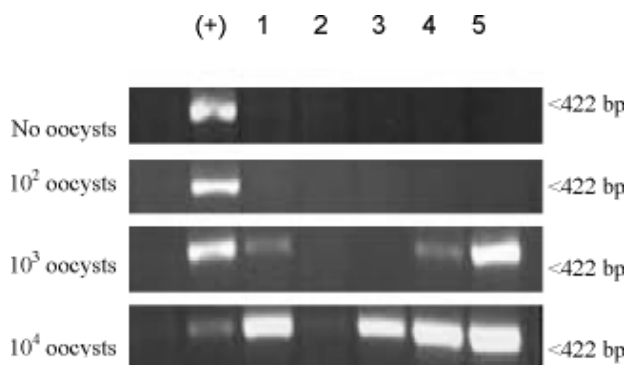


Figure 2 Gel electrophoresis analysis showing PCR products obtained from the uninfected and infected chicken receiving 100-10000 oocysts. The technique is applied on day4 after infection. (+) is a positive control of *Eimeria* DNA. Lanes 1-5 represent DNA extracted from tissues recovered from various parts of intestine (1) proximal, (2) upper middle, (3) lower middle, (4) distal part of small intestine and (5) a cecum.

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P39 THE DETECTION OF THE GYRA POINT MUTATION FROM QUINOLONE-RESISTANT *CAMPYLOBACTER JEJUNI* ISOLATED FROM BROILER INTESTINES

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Introduction

The treatment of human campylobacteriosis with quinolones and fluoroquinolones (FA) work by interfering with the DNA gyrase protein. The quinolone resistance determining region (QRDR) in the *gyrA* gene includes a major point mutation of codons 86 of threonine substituted by isoleucine (Thr-86-Ile), leading to the high level resistance to FA in *C. jejuni*¹. This study aimed to detect the frequency of Thr86 codon substitution in *gyrA* gene among *C. jejuni* isolates from the intestinal tract of broiler chicken, which are regarded as the reservoir of the infection, and also its association with nalidixic acid (NA) resistance.

Material and Methods

Bacteria and antimicrobial susceptibility testing

Twenty two *C. jejuni* strains were isolated from the intestinal tracts of broiler chickens taken from retail markets in and around Bangkok. The isolates were identified by the biochemical characteristics incorporated the protein profile by SDS-PAGE³. The antimicrobial susceptibility to NA (Oxoid) was performed by the disc diffusion method.

DNA preparation

DNAs were extracted by the instagene, as described by the manufacturer (BioRad).

Mismatch amplification mutation assay (MAMA)

The Thr86 mutations were detected by two pairs of primers, the first one to generate a 673 bp of *gyrA* gene, and the second one to detect the 265 bp of the Thr86-to-Ile mutation. PCR procedures were performed as previously described⁴.

Results and Discussion

The protein bands presented in the 22 strains of a species resembling to *C. jejuni* ATCC 33291 and distinct from *C. coli* ATCC 33539. One band of 47.17 kDa of *C. jejuni* as well as 56.37, 38.31, 30.05, and 28.43 kDa bands of *C. coli* were differentiated^{2,3} (Fig.1). The determination of the *gyrA* DNAs were shown among the *C. jejuni* isolates. The point mutation within Thr86 codon was also observed in all *C. jejuni* isolates, but the corresponding to NA resistance was shown only in wild types (Fig.2 and Table1).

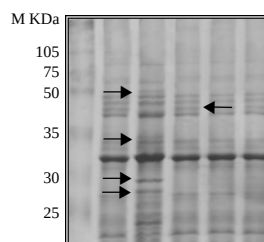


Figure 1 Protein patterns: lane1, Marker; 2 *C. jejuni* ATCC 33291; 3, *C. coli* ATCC 33539; 4-6 (wild types) B1, C2 and E., Arrowheads indicate characteristic bands for each species.

Table 1. Antimicrobial susceptibility of 22 wild strains of *C. jejuni* isolated from broiler intestines.

Names	Number	NA (30 g)	Thr86
A1-A4	4	R	+
B1-B5	5	R	+
C1-C11	11	R	+
D, E	2	R	+
<i>C. jejuni</i> ATCC 33291	1	S	+
<i>C. jejuni</i> ATCC 700819	1	S	-

R = resistant, S = sensitive

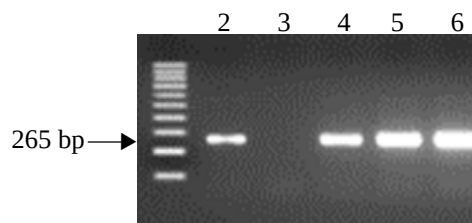


Figure 2 Lane1: 100 bp DNA ladder; 2, Ciprofloxacin-resistant *C. jejuni* ATCC 33291; 3, Ciprofoxacin sensitive *C. jejuni* ATCC 70081; 4-6, DNAs from Thr-86-to-Ile of NA resistant mutants, A1, B2, and C3 respectively.

The dissociation of those in *C. jejuni* ATCC 33291 may be caused by the low level of the MIC or borderline cases (ciprofloxacin, $R \geq 4 \mu\text{g/ml}$) resulting in NA sensitivity. This study demonstrated the wide use of quinolones and FA in chicken farms. High frequency (100%) resistance detected in broiler chickens is supposed to be a potential cause of the quinolone resistance problem in humans.

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P40 ANTIMICROBIAL SUSCEPTIBILITY OF STAPHYLOCOCCUS SPP. CAUSING SUBCLINICAL MASTITIS IN COWS FROM NAKHONPATHUM PROVINCE, THAILAND

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Introduction and Objectives

Antimicrobial therapy is a primary tool for the treatment and control mastitis in dairy herds. Staphylococci mostly cause subclinical mastitis. Antimicrobial susceptibility information will help guide the veterinarian in selecting the most appropriate antimicrobial agent for dry cow treatment. The purpose of this study was to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of selected antimicrobial agents against staphylococci causing mastitis in dairy cattle from Nakhonpathum province, Thailand.

Materials and Methods

The MIC and MBC of antimicrobial agents, including penicillin G, oxacillin, gentamicin, vancomycin, tetracycline, against 58 staphylococcal isolates causing subclinical bovine mastitis, was determined during 2003-2004, using a broth dilution technique, according to the National Committee for Clinical Laboratory Standards recommendation (NCCLS).

Result and Discussions

The results showed that the staphylococci causing bovine mastitis are resistant to penicillin G, oxacillin,

tetracycline, gentamicin and vancomycin, 69.2% (44/52), 4.7% (2/42), 29.4% (17/58), 11.1% (6/54), 2.0% (1/48), respectively (Table 1). There is a trend showing that more staphylococci isolates were resistant to penicillin G, gentamicin, and vancomycin than in our previous study. This information is useful as a background and guideline for monitoring antimicrobial susceptibility and choosing the best drug for the treatment of bovine mastitis in that area of Nakhonpathom province.

Acknowledgement

Faculty of Veterinary Science, Chulalongkorn University Bangkok Thailand, funded the study.

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Table 1. The distribution of MIC and MBC of selected antimicrobial agents against *Staphylococcus* spp. from mastitis (%)

Concentration		µg/mL									
		0.125	0.25	0.5	1.0	2.0	4	8	16	32	64
Penicillin	MIC	30.7*	11.5	5.7	1.9	7.6	3.8	15.3	23.0	.	.
	MBC	7.6	9.6	7.6	5.7	3.8	9.6	7.6	48.0	.	.
Oxacillin	MIC	38.1	26.1	9.5	9.5	4.7	7.1*	2.3	0.00	2.3	.
	MBC	0.00	4.7	7.1	11.9	21.4	30.9	2.3	4.7	16.6	.
Gentamicin	MIC	.	.	.	79.6	3.7	5.5*	3.7	1.8	5.5	.
	MBC	.	.	.	12.9	25.9	11.1	16.6	7.4	25.9	.
Tetracycline	MIC	.	.	62.0	5.1	3.4	0*	1.7	1.7	18.9	6.8
	MBC	.	.	1.7	5.1	8.6	12.0	12.0	13.7	13.7	32.7
Vancomycin	MIC	.	.	.	79.1	18.7	0*	0	0	0	2.08
	MBC	.	.	.	6.25	4.1	16.6	12.5	27.0	22.9	10.4

*Less than or equal to this concentration level considers as susceptible to antimicrobial agent.

P41 POLYMORPHISMS OF MICROSATELLITE MARKERS IN THAI DOMESTIC ELEPHANTS

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Introduction

Microsatellite loci which belong to Asian (*E. maximus*) and African elephants (*L. africana*) have been investigated^{1,2,3}. However, the number of loci reported is limited and, more importantly, the loci were tested in elephants from different parts of the world and may not represent the patterns of microsatellite loci in Thai domestic elephants. Thus, the determination and application of microsatellite markers for individual identification and parentage testing will further support conservation plans for elephants in Thailand.

Materials and Methods

Blood samples were collected from 16 elephants which belonged to 2 herds, one herd in Nakorn Pathom province (12 animals) and the other in Chonburi province (4 animals). DNA from each sample was isolated and microsatellite DNA were amplified by a polymerase chain reaction (PCR) using the 5 specific primers previously reported^{1,3,4}. The motif of each locus is shown in the following table.

LOCUS	MOTIF
LaT05	(CCAT) ₂ CCAC(CCAT) ₁₄ CAT(CCAT) ₁₇
LaT07	(ATCT) ₁₉ ...(ATCT) ₁₆ AT(CATC)15CATT(CATC) ₄
LaT16	(GGAT) ₃ GGCG(GGAT) ₁₈
LaT17	(GGAT) ₁₅ ...(GGAT) ₁₀
LaT26	(GGAT) ₃ G(GGAT) ₅ (GGGAT)7GGAT(GGGAT) ₃ CGAT(GGGAT) ₄

PCR products of 5 microsatellite loci were size-determined using polyacrylamide gel electrophoresis (PAGE) and analysis of allelic polymorphisms for each marker was performed in order to determine its suitability for individual identification and parentage testing.

Results and Discussion

Only four microsatellite markers LaT05, LaT16, LaT17 and LaT26 of the African elephants used in this study could be amplified. The sizes of the microsatellite

loci were determined to be 450-525 bp (LaT05); 300 bp (LaT16); 350 bp (LaT17); and 350-450 bp (LaT26) (Figs. 1).

The number of alleles and polymorphisms of microsatellite DAN in each elephant were determined and the number of alleles of LaT05, LaT16, LaT17 and LaT26 was 8 (a-h), 4 (a-d), 5 (a-e) and 7 (a-g) respectively.

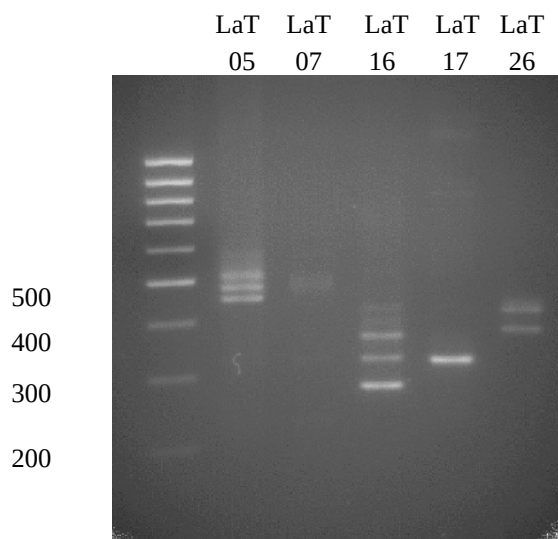


Figure 1 Size analysis of the microsatellite DNA.

The results of microsatellite analysis were in accordance with the pedigree data of the two herds of elephants, indicating that microsatellite loci LaT05, LaT16, LaT17, and LaT26 can be used for parentage identification in Thai domestic elephants.

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P42 AMMONIA MONITORING MEMBRANES: PROPERTIES AND FIELD TRIALS

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Introduction and Objectives

Ammonia is a common by-product of animal waste. Ammonia often accumulates inside poorly ventilated or poorly managed animal facilities. Elevated levels of ammonia can have a negative impact on animal health and production. Managing ammonia levels needs proper litter management but most of the available methods are not satisfactory.

Ammonia monitoring membranes are produced for practical application: they are convenient for the user and easy to interpret. The color of the membrane changes from yellow to purple, orange or green, depending on the ammonia concentration. In this report, some of the membrane's properties were studied in a closed system before being further investigated in a commercial poultry house.

Materials and Methods

The membranes were prepared by the Biochemistry Unit, Faculty of Veterinary Science, Chulalongkorn University, with 3 color ranges, yellow to purple (P membrane), yellow to orange and yellow to green. All three colors were mounted on a single frame.

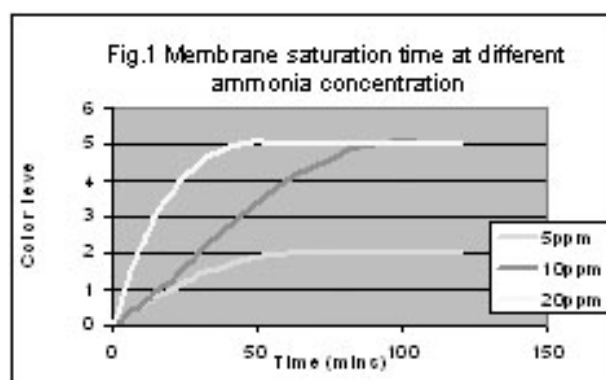
A closed chamber was made from clear acrylic, 0.5 x 0.5 x 0.5 m³. Ammonia was produced in the chamber by mixing 40%(w/v) sodium hydroxide with 10,000 ppm ammonium chloride solution. The ammonia concentration was measured by an electrochemical sensor (Oldham MX 2100) while the color changes were observed from outside the chamber.

To measure the ammonia concentration in an evaporated cooling system in a poultry house, the membranes were placed against the air flow for 1 hour, before collecting the data. The data was collected at between 60-90% humidity, a temperature of 28-35°C and a wind speed of 0.2-3.5 m/sec.

Results and Discussions

After exposure to ammonia, the color did not change immediately due to the gas penetration rate. The factors that induced color changes were therefore investigated.

First, a high concentration of ammonia induced the color reactions to change faster (Fig.1). Color changes reached a maximum level (purple) in 30 to 90 min, at an ammonia level of 20 and 10 ppm respectively. At 5 ppm, color could not reach the maximum level, and remained as a brown color due to the lower ammonia concentration. Second, higher humidity also reduced the saturation time (data not shown). All membranes demonstrated that different concentrations gave different colors.



The results on the studied Ammonia concentration measured by both sensor and membranes were compared. Membranes read 2-4ppm higher than the electrochemical sensor.

Range (ppm)	Sensor (ppm)	Membranes (ppm)	n
0-5	2.5±2	3.4±2.3	30
>5-10	9.1±1.1	11.2±2.3	23
>10-15	13.1±1.5	15.6±3.6	41
>15-20	17.6±1.6	19.6±4.1	34
>20	23.2±2.3	24±1.8	20

It should be noted that the nature of both methods are different. Sensors are quantitative and very sensitive to any fluctuation caused by air flow while membranes are semi quantitative and less sensitive.

Reference

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