

R&D Highlights

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PCC's continuing R&D efforts

By JOAHNA G. GOYAGOY

The Philippine Carabao Center (PCC) conducts an annual research and development (R&D) review as part of its continuing commitment as a premier institution for livestock biotechnology.

The following are completed researches in the fields of animal nutrition, animal health, breeding and genetics, dairy products and socio-economics presented during the previous R&D reviews at PCC.

Animal Nutrition

Topping the list are the studies on "Comparative Performance of USM Buffalo Herd on Milk Production under Two Different Feeding Managements" and "Effects of Improved Nutrition on the Recovery of Lactating Cows to Shorten Calving Interval and Increase Calf Productivity (Using Cow Calendar as a Monitoring Tool).

The first study highlighted the potential of increasing the milk produce of cows fed with a combination of grass and legume

forages compared with the traditional feeding practice (pure grass feeding). The second implied that employing the breeding calendar method can aid in determining the nutritional intervention for calf drop thereby improving its production performance. The results also showed that the calving interval was shortened from 26.94 months to 18.18 months.

Animal Health

Among the valuable researches under animal health is the "Development of DNA-Based Methods for Rapid and Safe Screening of Bull Semen for FMD Virus Infection." This research study demonstrated the use of nucleic acid-based detection of FMD virus without isolation and culture of the live virus thus, it is considered safe. The study also demonstrated the possible use of inactivated and purified vaccine for RNA (ribonucleic acid) analysis to generate an external positive control for Reverse Transcriptase-Polymerase



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multi-species of animals raised in the farm should also be considered because cross infection can occur. Finally, *Cryptosporidium spp.* infection can be included as a possible cause of diarrhea among farm animals in the province. Hence, the provincial and rural health offices of the province should be notified and be aware of such risks.

The results on the second research paper, on the other hand, showed that the occurrence of cryptosporidiosis among

the dams and calves in the PCC gene pool are not related to gender, birth weight, and parasitologic state of the dam after delivery. Findings revealed that the transmission of infection could be traced to the single animal caretaker who mechanically transferred the oocysts through contaminated hands during assistance in calving and feeding of calves.

Breeding and Genetics

A study on “DNA-Based Detection of Major Mastitis-Causing Pathogens in Buffalo Milk,” was conducted at the PCC-National Headquarters which showed the feasibility of using multiplex PCR (mPCR) method in identifying the pathogen causing mastitis, one of the major diseases in the dairy sector. The tested milk under mPCR was compared with the results derived from bacteriological culture. The findings demonstrated that mPCR promises a sensitive, rapid and specific test for the improvements in the disease control and accurate decision for treatment.

The second study is titled “Molecular Characterization of the Philippine Carabao (*Bubalus bubalis L.*) from the Major Island Groups of the Philippines Using Molecular Cloning and Sequence Analysis of the D-Loop of Mitochondrial DNA” conducted at the PCC-UP Los Baños.

The study used blood samples from the major island groups of the Philippines specifically in Cagayan, Batangas, Bohol, and Bukidnon.

The PCR products were cloned and sequenced. The result of the study recognized the close relationship of the Philippine Carabao to the Chinese

A strategic R&D planning was conducted after the in-house review of PCC’s completed and ongoing researches last May 28. The seminar-lecture was given by Dr. Synan S. Baguio (inset photo), assistant director of Livestock Research Division Philippine Council for Agriculture, Fisheries and Natural Resources Research and Development (LRD-PCARRD).

Chain Reaction (RT-PCR) and Reverse Transcriptase-Loop-Mediated Amplification (RT-LAMP). It showed that RT-LAMP assay is more advantageous due to its simplicity, rapid and easy detection which suggests that it can be used for field deployment.

Another study titled “Virulence and Genetic Variability of *Trypanosoma evansi* Isolated from Animals in The Philippines.” This research study assessed the effect of the isolates to the host by determining parasitemia, body weight changes, and host survival using a murine model.

T. evansi is the causal agent of the widespread surra which is also considered as one of the most economically important animal parasitic diseases in the Philippines. The study assessed the effect of the isolates to the host by determining parasitemia, body weight changes, and host survival using a murine model.

Two studies were also completed dealing with *Cryptosporidium spp.* which are “Detection of *Cryptosporidium spp.* Infection among Swamp Buffalo in Selected Municipalities of Aurora Province” and “Incidence and Risk Factors of *Cryptosporidium spp.* Infection in Neonatal Calves at PCC Gene Pool.”

The first one is an epidemiological study conducted to detect the occurrence of *Cryptosporidium spp.* infection in smallhold farms raising swamp buffaloes in selected municipalities of Aurora province namely Baler, San Luis, and Maria Aurora. It highlighted the importance of hygienic practices among animal handlers to minimize risk factors of transmitting infection to other susceptible animals. Since cryptosporidiosis affects a wide range of hosts including man,

buffaloes. This result may be helpful in the development of species identification and differentiation methods.

Dairy Products

Recent advances in dairy products are making inroads to expanding healthy food choices and oasis of daily cash flow for most rural dairying communities. Adding to these is the potential for locally produced products entering into the national market. Along this line, two studies were completed at the PCC-Visayas State University.

The first study titled “Production and Quality Evaluation of Nata from Buffalo Milk Whey” showcased the possibility of using whey, a by-product in the production of cheese, as a fermenting medium in the production of nata.

The second study titled “Evaluation of the Sensory Quality and Acceptability of Soft White Cheese from Buffalo’s Milk as Influenced by the Levels of Salt and Acid,” explained that the levels of salt and acid only affected the texture of the product but had no influence on its color, saltiness, sourness, and general acceptability. Based on the results, the most acceptable formulation is 2% (w/w) salt and 4% (w/w) acid.

Socio-Economics

As part of ensuring the development and improvement of the projects and programs of PCC, studies on socio economics are seen necessary.

In this light, two researches were completed at PCC-Ubay Stock Farm and PCC-Don Mariano Marcos Memorial State University.

The study on “Factors Associated with the Performance of Artificial Insemination in Caracows in the Impact Zone of PCC, Bohol: A Matched Case-Control Study” stressed the importance of proper handling of caracows after undergoing AI. The results of the study further demonstrated that caracows should be allowed to wallow and rest after AI and stressed that AI is best conducted in the afternoon.

[On the cover: A pool of PCC’s promising young scientists who are contributory to PCC’s 17 years of excellence in R&D. From front clockwise: Dr. Rubigilda Paraguison, Amie Villanueva, Mark Francis Soliven, Dr. Jesus Rommel Herrera, Dr. Roxan Grace Cacho, Leslie Anne del Barrio and Jennifer Maramba.]

DNA-based method is proving effective, rapid for FMD detection

BY JOAHNA G. GOYAGOY

The Philippines seriously pursues to acquire certification to become a foot-and-mouth disease (FMD)-free country without vaccination. The PCC research team is making headways to protect the livestock from suspicious incoming animals and germplasms such as semen and embryo.

FMD is an acute and highly transmittable disease which affects mainly cloven-footed animals such as carabao, goat and sheep. Transmission occurs through inhalation of droplets and ingestion of infected food, inoculation with contaminated virus, contact with contaminated clothing, veterinary instruments and insemination with contaminated semen. This disease can be economically devastating due to its rapid transmission and effect in livestock production.

Artificial insemination can also be a means of disease transfer if the semen is contaminated with virus. FMD virus spread can be extensive since it can affect numerous farms, areas or countries within a short period of time. Therefore, there is a need to ensure the safety of the samples such as semen for artificial insemination with the help of a reliable, rapid and sensitive test.

A particular research study was intended to advance the molecular diagnostic tools for Philippine Carabao Center research laboratory to come up with reliable and rapid detection of the Foot-and-Mouth disease virus.

The team of the PCC Molecular Genetics Laboratory, led by Dr. Rubigilda C. Paraguison, focused on the use of RNA (Ribonucleic acid) extraction from purified vaccine against FMD and frozen semen samples using RT-PCR and RT-LAMP. Dr. Paraguison put forward a research paper entitled “Development of DNA-based methods for rapid and safe screening of bull semen for FMD virus infection”. This study demonstrated the detection of the virus in semen at a high level of sensitivity through the use of reverse transcriptase-polymerase chain reactions (RT-PCR). This diagnostic

method is known to be very sensitive, specific and rapid detection of viral genomic sequences. It also demonstrated the possibility of using inactivated and purified vaccine for RNA analysis to generate an external positive control for RT-PCR assay. And this suggests the possibility of using other killed virus vaccines as an external positive control for molecular-based detection of other viral diseases. It was also demonstrated that a more recent method called Reverse Transcriptase-Loop-Mediated Amplification (RT-LAMP) detects the virus without the use of complicated equipment such as thermal cyclers thus it can be used for field deployment. RT-LAMP can also be used to detect various infectious agents and the results can be obtained in an hour or less, which makes virus detection faster.

“LAMP” which stands for Loop-mediated Isothermal Amplification is a simple, rapid, specific and cost-effective nucleic acid amplification method. It is characterized by the use of four different DNA markers or primers specifically designed to recognize six distinct regions on the target gene and the reaction process proceeds at a constant temperature using strand displacement reaction. Amplification and detection of gene can be completed in a single step, by incubating the mixture of samples, primers, DNA polymerase with strand displacement activity and substrates at a constant temperature (about 65°C). It provides high amplification efficiency, with DNA being amplified 90-100 times in 15-60 minutes. Because of its high specificity, the presence of amplified product can indicate the presence of target gene.

Accordingly, once established, this technique can be considered as a standard for diagnosing any viral diseases such as Bovine Viral Diarrhea Virus (BVDV) and encephalomyocarditis virus (EMCV).

While RT-LAMP presents many advantages over RT-PCR, it is quite new in diagnostic laboratories, thus the

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Improved DNA-based method readily detects mastitis-causing pathogens in carabao's milk

BY ROWENA G. BUMANLAG

An improved Polymerase chain reaction (PCR) method, called multiplex polymerase chain reaction (mPCR), is capable of rapidly and simultaneously detecting four common mastitis-causing pathogens in milk, recent studies of PCC showed.

The PCR is a molecular biology technique to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

The mPCR, a modification of the basic PCR process, allows sensitive and synchronized screening of multiple pathogens in just a single assay based on amplification of bacterial DNA.

Another significant advantage of using mPCR, the study showed, is the cost-effectiveness of the method compared with simplex PCR which requires reagents such as the extensive Taq DNA polymerase. It also requires less preparation and time for analyzing mastitis-causing agents since mPCR can detect more microorganisms in one reaction whereas, simplex can only detect one.

The National Mastitis Council describes mastitis as an inflammation of the

mammary gland that commonly infects dairy cows causing economic loss in dairy industries due to reduced production, discarded milk, costly drug therapy, veterinarian fees, premature culling, and increased labor expenses.

Mastitic milk has high somatic cell count resulting in shortened shelf life and low quality which consequently makes the milk unsuitable for consumption. Thus, early and rapid detection of causative agent is important to employ appropriate treatment and prevent further losses.

Studies showed that conventional microbiological methods of identifying mastitis-causing pathogens such as bacterial culture methods are time-consuming and generally require more than 48 hours to identify the causative agent of the disease.

Several disadvantages associated with current microbiological methods were also observed. These included resulting negative culture caused by residual antibiotics following antibiotic therapy or by the low number of pathogens detected in the sample.

The research study conducted by PCC molecular biologist Dr. Rubigilda Paraguison and co-researchers tried the application of mPCR and proved its efficiency in detecting major

pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. These pathogens were directly tested from DNA isolates of carabao's milk.

Dr. Paraguison said, "This study suggests that mPCR can be a practical tool applicable in molecular dairy laboratories in the Philippines for rapidly diagnosing mastitis-causing pathogens in milk that would make significant improvements in the disease control and accurate decision for treatment."

She added, "We have optimized a protocol for DNA isolation and multiplex PCR-based detection of major mastitis-causing pathogens applicable for screening raw buffalo milk. The test was directly performed from milk samples without microbiological culture step and specific for the target strains of bacteria."

With the positive results of the study, the research team aimed to advance diagnostic strategies for the fast, efficient and cost-effective detection of mastitis-causative agents using mPCR, which would later on improve disease control and treatment protocols for mastitis.

The research work was carried out by the PCC and partly funded by the Korea International Cooperation Agency.

The results of their study confirmed other studies which showed that nutritional treatment affects the reproductive performance of dairy buffaloes.

According to the experts, higher conception rate and a decreased incidence of anaestrus and cystic ovulatory disease of dairy buffaloes were noted due to this nutritional intervention.

The team found out that the calving intervals of the dairy buffaloes in their first, second and third calvings were 26.94 months, 20.08 months and 18.18 months, respectively. Research findings showed that the difference in the three calvings was statistically significant ($P < 0.01$).

It was noted that a decline of 6.86 months

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Enhanced feed values shorten calving interval, improve calf production

BY KHRIZIE EVERT M. MARCELO

Dairy farmers can reduce the calving interval of their dams by improving nutritional intake aided by the cow breeding calendar system, results of studies showed.

Researchers, Virgilio V. Lopez, Mary Joy S. Paman, Freddie F. Justo and Benjamin John Basilio of PCC at University of Southern Mindanao (PCC-USM)

examined the management practices usually applied in lactating buffaloes. The study was conducted from April 2005 to March 2009.

By implementing proper nutrition, monitoring of reproductive status with the use of the calendar and fertility chart, proper management after calving and management of post-partum of buffaloes, the performance of the buffaloes improved and their calving interval was shortened, they said.

Where the native carabaos came from

By KHRIZIE EVERT M. MARCELO

It is a given fact that the Philippine carabao is not endemic to the country but was brought to the Philippine shores by the early migrants several centuries back.

The carabao (a swamp buffalo) is regarded as the country's national animal. It is typically associated with the farmers as their draft animal of choice from plowing the rice fields and a handy animal for many other uses.

But what is the lineage of the Philippine carabao? Which type of water buffalo is it closely related to?

Genetic characterization can provide the answer to these questions. Experts said that it can provide means in determining the genetic diversity within species, enhance their genetic component, and uphold the animal's conservation.

An effective way to measure the genetic link among these species is through the use of the D-loop mitochondrial Deoxyribonucleic acid (mtDNA), microsatellites and protein coding loci.

The mtDNA, is a vital tool in the study of intra and interspecific variation due to its maternal transmission, no recombination and high substitution rate. It is also valuable in establishing phylogenetic and relationship studies among related populations.

Leslie Anne Del Barrio of the Philippine Carabao Center (PCC) and her co-researchers conducted a thesis study aimed specifically to characterize the Philippine carabao at the molecular level through mtDNA analysis, to analyze its mtDNA sequences and to compare them to the sequences from different parts of the country and from other countries.

Del Barrio and her team, through mtDNA analysis, found that the Philippine carabao descended from the maternal line of Chinese buffaloes.

The team acquired mtDNA d-loop from 36 blood and DNA samples

using molecular cloning and sequence analysis to obtain knowledge on the origin and genetic diversity of the Philippine carabao. The samples were gathered from the animals in different major island groups of the country particularly Cagayan, Batangas, Bohol and Bukidnon.

From the samples collected, DNA was extracted and the D-loop region was amplified through polymerase chain reaction (PCR) primers CB3 and A.dLo.H with PCR product of 484 -bp. The D-loop PCR products were cloned and sent to Macrogen for sequencing.

To measure the accuracy of the analysis, computer programs and databases, such as the GenBank, BLAST programs, Vector NTI suite, Chromas 2.33, ClustalX and TreeView, were used to examine the obtained sequences.

A total of 97.5% homology was detected from the multiple sequence alignment analysis of the samples. It was established that they are highly homologous.

According to the Blast-N database, the sequences showed 99-100% compatibility from various Chinese buffalo breeds. This implies that the Philippine carabao descended from these maternal lines.

Another interesting result is that when a rooted phylogenetic tree was created using the mtDNA samples, it confirmed that the Philippine carabao belongs to Lineage A of swamp buffaloes and that it is closely related to the Chinese buffaloes.

The findings support the hypothesis of Lau et al. (1998) and Kierstein et al. (2004) that the buffaloes from the Philippines came from China.

The researchers reported that similar studies of the sequence analysis of the mtDNA of the Philippine Carabao were done before. Scientists from Malaysia, Australia and Japan previously sequenced the D-loop region and cytochrome b gene and mtDNA of the Philippine carabao.

The data for these sequences, however, were not available in the Genbank,



the genetic sequence database of National Institutes of Health (NIH) where annotated collection of all DNA sequences is publicly available.

The analyzed sequence obtained in this study may be helpful for future reference on other molecular studies, specifically in sequencing the whole mtDNA genome of the Philippine carabao, the researchers said.

The prepared clones are also vital in the development of species identification and differentiation methods. It will be stored and can be retrieved for future use.

Researchers said that their study can be of use in designing proper animal breeding programs and in conserving the different genetic lineages found in the country.

DNA-based method...

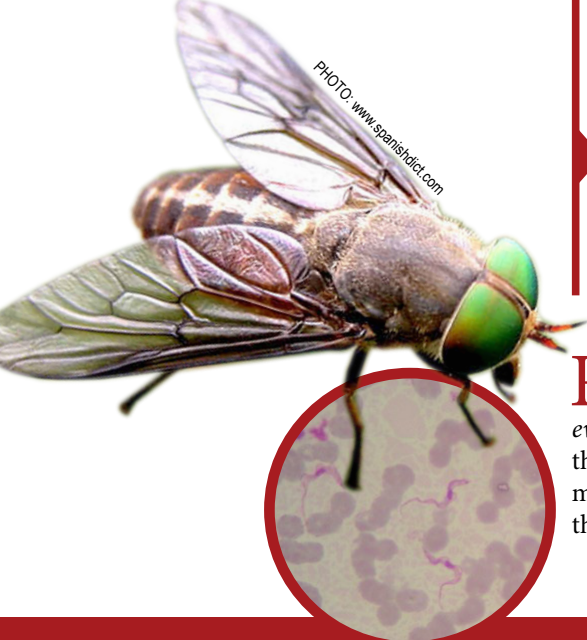
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possibility for using it in the Philippine setting has not yet been tested.

"It is still imperative that protocols be observed for each identified disease in a research facility before subjecting these viruses in field laboratories," Dr. Paraguison said.

The research team recommended that since RT-LAMP is a new FMD virus detection method, it is best that this would be run in parallel with the established RT-PCR assay as reference test.

While expensive enzyme-reagents might get in the way, using nucleic acid-based test is seen as a preventive strategy and a more practical way to save millions of peso in protecting livestock species against the dreaded FMD.



By JOAHNA G. GOYAGOY

Identification of *T. evansi* genetic diversity vital to surra prevention

Recent development in identifying the genetic diversities of *Trypanosoma evansi* isolates among swamp buffaloes in the Philippines indicated significance for more effective control and management of the disease.

This finding was a result of the study by Ms. Marjo Villareal and Dr. Windell L. Rivera of the University of the Philippines-Diliman together with Dr. Claro N. Mingala of the Philippine Carabao Center (PCC).

Trypanosoma evansi is a protozoan hemoflagellate which causes surra, one of the most economically important diseases of buffaloes in the Philippines. This is also endemic in countries such as Africa, South and Central America, and Asia.

Surra disease posts high risk in domestic animals

Trypanosoma evansi (*T. evansi*), the causative agent of surra disease, posts high health risk to domestic animals in Asia, including the Philippines, PCC research findings showed.

Surra is believed to be one of the leading diseases that cause buffalo deaths due to progressive weight loss. It also causes fever, weakness, and anemia. It is transmitted by biting insects such as big flies (*Tabanus lineola*), which may cause abortion on the late trimester of pregnancy resulting to decrease in reproductive efficiency of dairy animals.

This disease is endemic in many parts of Africa, Asia, and South America and proved to be fatal to thousands of animals mostly during disease outbreaks. In the Philippines, surra is considered as one of the most critical animal parasitic diseases causing great damage to the livestock economy.

A study conducted by a group of students from the University of the Philippines-Diliman, made use of BALB/c mice, to test the rate of infectivity of *T. evansi* from the suspected infected water buffalo from selected sites in Luzon, Visayas, and Mindanao. BALB/c mice is an albino, laboratory-bred strain of the house mouse which are among the most widely used inbred strains used in animal experimentation.

The blood samples collected from infected and non-infected water buffaloes for the research were introduced to mice which were approximately eight weeks old with

weight ranging from 17-35 grams.

Trypanosomes are protozoan parasites that can infect humans and livestock. *T. evansi* is one of the most common and widespread pathogens of this kind. It has a broad host range including domestic and wild mammals.

The ability to characterize stocks and strains of protozoa is fundamental to determine the frequency and distribution of disease and measure risks.

Employing murine model, comparisons showed that from the collected buffalo blood samples, all the infected mice died before the 16th day. Samples from Visayas showed the most rapid death occurrence (5th -7th day) compared to the samples from Luzon (8th day) and Mindanao (8th-15th day).

Murine model used for trypanosomiasis is most popular for research purposes because of its advanced logistics in determining virulence rate.

The BALB/c mice, used as murine models, consistently recorded fatal reaction resulting to congestion, haemorrhage, presence of parasite in the portal tract and fatty changes of the liver. This showed that the infection of this virus rapidly occurs resulting to great economic losses.

However, while murine models can determine virulence variability among different isolates and histopathological changes due to *T. evansi* infection, the present study showed that BALB/c mice, as murine model, are not ideal to quantify all the major clinical symptoms of surra disease because of its small size.

Aside from weakness, fever and anemia, *T. evansi* infection also causes progressive weight loss leading to death which eventually causes great economic loss.

Identifying genetic diversities is significant in determining the epidemiology or how the disease often more pathogenic in animals. It also gives guides for disease prevention and management.

To identify the genetic diversities of *T. evansi*, isolates from swamp buffaloes in the Philippines were investigated through Polymerase Chain Reaction (PCR) and deoxyribonucleic acid (DNA) sequencing.

Employing these kinds of molecular tools, the research indicated that isolates gathered from Luzon and the Visayas have similarities with the isolates from Mindanao.

These differences and similarities, however, can be attributed to the differences in insect vectors, host species infected, and the geographical location of the isolates, the study explains.

Further, the result also indicated clustering of single lineage among the isolates used. Subgroups were also observed, however, the research team recommends that more isolates are needed to further identify the subgroups of *T. evansi*.

While incidence of surra continues to post threats to the animals, more studies on identifying the genetic diversities of *T. evansi* are seen important to further manage and prevent the spread of this disease.

Biotech scientists prove vitrification is better cryopreservation method for buffalo oocytes

By ROWENA G. BUMANLAG

Vitrification is a better method in the cryopreservation of in-vitro matured oocytes and produces significantly higher normal oocytes in terms of morphology and subsequent development.

This conclusion was drawn in a recently completed research study on the cryopreservation of in-vitro matured buffalo oocytes by slow freezing and vitrification methods. The study was conducted by a team of biotech scientists led by Dr. Eufrocina Atabay of PCC's reproductive biotechnology laboratory with Dr. Edwin Atabay, Flocerfida Aquino, Dr. Danilda Duran and Rodante de Vera, as co-authors.

Vitrification, studies show, uses a high concentration of cryoprotectant and rapid cooling rates to solidify solutions. It is also faster and less expensive compared to slow freezing.

In the conventional slow freezing system, using a programmed freezer, cooling rates are controlled to allow extracellular and intracellular water exchange without serious change in cell shape. This method, however, yielded unsatisfactory results when used for cells that are sensitive to chilling, such as oocytes.

Cryopreservation, or cryogenically freezing, is described as storing a living organism to a low sub-zero temperature (-196°C) or the boiling point of liquid nitrogen and revive it at the same state for future use.

In the paper titled "Prospects of Livestock Biotechnology in the Philippines", PCC executive director Dr. Libertado Cruz said that cryopreservation of oocytes is one of the major efforts on livestock biotechnology research and development in the country and is currently being refined due to demand for oocytes for use in in-vitro embryo production (IVEP).

He further said that the objective of cryopreservation of oocytes is to obtain considerable number of oocytes from all possible and practicable sources for the

widescale IVEP of water buffaloes and other ruminants as well.

In the study of Dr. Atabay and her team, they endeavored to establish the protocol for the cryopreservation of in-vitro matured oocytes collected from buffaloes for use in various researches.

"The development of an efficient oocyte cryopreservation protocols will widen and improve the strategic implementation of various reproductive technologies for genetic improvement of the buffalo species. The technique will facilitate exchange and transport of germplasm among countries with fewer requirements compared to importing live animals," Dr. Atabay said.

Slow freezing and vitrification methods were compared in terms of morphological survival after freezing, developmental ability after fertilization and subsequent embryonic culture in-vitro.

Results showed that matured buffalo oocytes cryopreserved using vitrification method had higher viability rates at post-warming compared to conventional slow freezing.

This variation was due to the faster cooling rate possible in vitrification method. At approximately 2500°C per minute, the high cooling rate permits oocytes to pass through critical temperature zone quickly which decreases cryoinjury.

In slow freezing method, intracellular ice formation can occur, resulting in cryoinjury and cell death.

Evaluation of the post-thaw morphology of frozen oocytes was done using an inverted microscope and were assessed based on their normal or abnormal state. Normal oocytes have spherical and asymmetrical shape with no signs of lysis, membrane damage, swelling, vacuolization and degeneration or leakage of the cellular content. Abnormal oocytes have ruptured zona pellucida or ruptured vitelline membrane and fragmented



Oocyte evaluation and classification using stereomicroscope

cytoplasm with signs of degeneration.

Generally, a cleavage rate of 20.33 percent was observed after in-vitro fertilization (IVF) of vitrified oocytes with some embryos reaching morula and blastocyst stages.

Although a proven more effective method in freezing buffalo oocytes, vitrification is associated with hardening of zona pellucida, resulting in low fertilization of morphologically normal oocytes.

Thus, the study recommended intracytoplasmic sperm injection (ICSI) as an alternative approach of fertilization by sperm to maximize the use of vitrified oocytes for IVEP. ICSI is an assisted reproductive technique (ART) where a single sperm is mechanically injected into an egg cell.

The research study was co-funded by PCC and the Bureau of Agricultural Research (BAR). It was supported by the Philippine Council for Agricultural Resources Research and Development (PCARRD) through the project "Cloning by Somatic Cell Nuclear Transfer as a Tool for Genetic Improvement in Water Buffaloes".

Risk factors causing cryptosporidiosis among swamp buffaloes detected; solution to problem suggested

By KHRIZIE EVERT M. MARCELO



Cryptosporidium is a protozoan parasite that causes cryptosporidiosis, also known as “crypto”. It lives in the intestinal tract of infected animals. An infected animal could release millions of crypto parasites in a bowel movement.

It is one of the most common waterborne diseases. The infection could spread out, once ingested, through contaminated areas like soil, bodies of water and uncooked food that has been exposed with the feces of infected animals.

In a recent study conducted by Shierlene Rose P. Quiamco of the College of Veterinary Science and Medicine, Central Luzon State University (CVSM-CLSU), in collaboration with the Philippine Carabao Center (PCC), the prevalence of cryptosporidium among swamp buffaloes in selected municipalities of Aurora was detected using microscopy.

The study shows that the occurrence of this disease was detected from 64 out of 146 heads or 44% of swamp buffaloes in smallhold livestock farm in selected municipalities of Aurora province from May 2007 to April 2008.

Quiamco examined fecal samples from 146 buffaloes in 86 farms from Baler, San Luis and Maria Aurora.

These samples were processed by means of fecal concentration method and fecal sediment staining using the Kinyoun’s Acid Fast, a method of staining acid-fast microorganism that helps reveal the presence of cryptosporidium.

Results showed that through microscopy, the *Cryptosporidium spp.* oocysts when stained by Kinyoun’s Acid Fast, appeared magenta red in color and measured 4 to 5 micrometer (um) under oil immersion (x100).

According to the researcher, out of 146 swamp buffaloes, Baler got the highest prevalence with 48% that is, 12 out of 25 swamp buffaloes were tested positive. On the other hand, San Luis and Maria Aurora got 45% (18 out of 40) and 42% (34 out of 81), respectively.

The owners of 86 farms were likewise interviewed. Twenty seven farms from 10 barangays in San Luis, 15 from four barangays in Baler and 44 from 15 barangays in Maria Aurora. Fifty-two or 60.5% of these farms tested positive for *Cryptosporidium* infection.

Animal raisers who were interviewed said that based on their experiences, the following were the risk factors in the possible transmissions of *Cryptosporidium*

spp. in swamp buffaloes: association of proper medication administered against diarrheal cases (most common sign of intestinal protozoa infection) among animals in the farm; direct access of swamp buffalo to fecal-contaminated drinking water; presence or absence of excreta in the vicinity; and acceptability of drinking water source in the farm; the proper disposal of animal and human waste in the farm; proper garbage disposal in the farm; access to water sources such as creeks, rivers, springs and irrigation canal to drink, wallow and defecate; and, presence of stray cats and rodents in the shelter and silage area of the farm.

The gathered data from the questionnaires were tabulated and the researcher found out that 75% of the selected farms were exposed highly to risk factors such as manner of excreta disposal, visible excreta and abundant flies, manner of garbage disposal, access to natural bodies of water and presence of animal scavengers in the farm.

Among these factors, the risk in the manner of garbage disposal was found to be connected with oocyst density among infected swamp buffaloes at 20% level of significance.

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AI-treated caracows demand proper handling, more care from farmers

BY JOAHNA G. GOYAGOY

Improper handling of caracows after subjecting to artificial insemination (AI) may cause failure of fertilization to produce calves, results of studies indicated.

By considering the proper time of the day for AI activity, letting the caracows stay in the AI site before walking it home or to another place, and allowing the caracow to wallow after AI, will give more chance for AI to succeed.

Researchers J. G. Polestico, E. B. Lañada and Caro B. Salces of Philippine Carabao Center at Ubay Stock Farm (PCC-USF) in Bohol assessed the management activities of the caracow owners and AI technicians at the impact zones in Ubay and Mabini. The caracow referred in the study was a mature female carabao that already gave birth.

The study, was meant to determine the factors associated with the performance of AI in caracows using a case-control study. It was found out that AI in caracows which already had offspring was more successful than those caracows which were subjected to AI for the first time.

In relation to this, a similar study was conducted using match-cased control analysis to determine the factors of the success and failure of subjecting AI to caracows in management-related activities.

Best time for AI treatment

Research findings indicated that buffaloes have a specific time of estrus or diurnal trend which mainly occurs between 6:00 pm fertilization to produce calves, according to the research study findings, and 12 midnight.

The most receptive time of female buffaloes to mating usually lasts for 18.5 to 24.8 hours. Since the best time to inseminate to achieve maximum fertility is about 12 to 18 hours before ovulation, or about 18 hours after the onset of

estrus, it is best to follow this biological phenomenon as a guide.

Moreover, high temperature delays or prevents ovulation. AI should take place in the afternoon because the maximum fertility of caracows mostly falls on this time. In addition, ambient temperature and air humidity are better in the afternoon than during late morning.

Wallowing of caracows after AI

Twenty-two percent of caracows subjected to AI were not allowed to wallow which have caused the failure of insemination. Caracows when exposed to high temperature or above 28°C at the time of breeding and for 72 hours thereafter failed to conceive, the study said.

Since the biological characteristics of the animal require cooling, they should be given special protection especially during summer season. They should also be provided with clean wallows and if not available, should be treated with cold showers daily during hot weather.

It must be understood that water buffaloes as semi-aquatic animals are not heat tolerant. Research says that water buffaloes, after given AI, should be allowed to cool off through wallowing to cope with the heat since buffaloes have few sweat glands. Allowing the caracow to wallow after AI permits greater percentage of successful insemination as it relieves the animal from stress, the study said.

Caracow rest

To avoid further stress, which causes reproduction failure, the caracows should be given enough time to rest after being inseminated. Before bringing the inseminated caracows home or to another place, the farmer should let them stay at the AI site to rest and to avoid stress from heat especially if insemination was done near noon.

Proper handling of artificially inseminated animals optimizes the benefits of this technology, the study concluded.

Enhanced feed values...

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was monitored from the first calving to the second calving and 1.9 months between the second and third calvings.

T-test was employed to examine the differences among the three calving intervals.

The researchers said that the cow calendar served as a tool in the daily monitoring of each buffalo specifically after giving birth, thus, keen observation of the expected time of estrus after calving is possible, allowing artificial insemination to likely succeed.

The feeding scheme of these animals was also changed. Instead of feeding them napier and corn silage, it was altered with 70% grass (napier and corn) and 30% legumes (rensonii) in a cut-and-carry system.

According to experts, it is essential that dairy buffaloes have good body conditions at calving and at early lactation because their food consumption decreases during these periods.

Further, insufficient conversion of energy may cause a delay in the continuation of reproductive functions, resulting in fertility reduction, hence, increasing the calving interval.

The researchers concluded that these management practices are useful in enhancing the production performance of the animals.

They suggested that providing good nutrition should be a priority activity by the dairy farmers in their dairy buffaloes. They recommended the holding of gynecological examination before and after calving to immediately address reproductive tract abnormalities.



Buffalo milk whey is good alternative culture medium for nata production

BY ROWENA G. BUMANLAG

The generally wasted liquid residue resulting from cheese production can be a very good culture medium in nata production, a recent collaborative research study of the Visayas State University (VSU) and the PCC at Visayas State University (PCC-VSU) revealed.

Whey protein is known to possess the highest biological value (BV) among the dietary proteins. This is because whey contains readily absorb proteins. These are used in protein synthesis, which is a vital mechanism in body growth and changes. Moreover, whey has high concentrations of essential amino acids which make it an ideal addition to other protein sources such as vegetable proteins.

Apart from being a culture medium for nata, whey has several other uses such as animal feed supplement, baking ingredient, supplement for Type 2 diabetics as whey can help regulate and reduce spikes in blood sugar levels through increased insulin secretion and as an ingredient for post-workout refreshment, such as shakes, by individuals who want to gain muscle mass, among others.

There are two types of whey: acid whey produced from cottage cheese manufacture and sweet whey which results from the production of rennet-coagulated cheese.

Nata de coco, on the other hand, is a popular delicacy used as a dessert and

an ingredient in ice cream, yogurt, fruit cocktail and a mainstay in the popular refreshment called halo-halo (fruit-mixed cooler). It is generally chewy, translucent and jelly-like and is produced from the fermentation of sugar and other components by gram negative, cellulose-synthesizing bacteria called *Acetobacter xylinum*.

The study was conducted by Charina Javier and Dr. Roberta Lauzon from the Department of Food Science and Technology of the VSU College of Engineering and Agri-Industries in collaboration with the Philippine Carabao Center.

It aimed to produce nata using buffalo milk whey from white cheese manufacture as a culture medium, determine the effects of acidity and sugar levels on the quality of nata produced from milk whey, determine the physico-chemical characteristics of the nata produced, evaluate the sensory qualities of the resulting nata, determine the cost of production, and determine the optimum conditions required to produce nata using milk whey.

The study used a 3x3 factorial design with nine treatments to evaluate the effects of acidity and sugar levels to the nata end-product using Response Surface Regression (RSREG) analysis.

Physico-chemical properties such as pH, total soluble solids (TSS) and titratable

acidity (TA) of the end product were determined.

Sensory characteristics, which include overall acceptability, color, firmness, aroma and flavor, were also evaluated following standard procedures.

Results showed that the sensory characteristics of the nata produced out of milk whey were not significantly affected by some of the parameters analyzed except TA, thickness and growth rate. The study further revealed that nata can be produced from milk whey even without adding acid due to the addition of acid into the milk during cheese production.

Likewise, result of the production cost analysis indicated that 110 grams of nata costs P35.70, which is within the acceptability area when analyzed using RSREG.

Although the study proved that milk whey or residue from kesong puti (white cheese) production can be utilized as a fermenting medium to produce nata, the researchers recommended further studies on assessment of the effect of acidity and sugar levels on the quality of nata, comparison of the quality of milk whey nata with nata de coco as the control, evaluation of the complete physico-chemical properties of the product and conduct of consumer evaluation.

The study was co-implemented and funded by VSU and PCC at VSU.



Study recommends 2:4 salt and vinegar ratio for soft cheese-making

By ROWENA G. BUMANLAG

Kesong puti or keso (soft cheese) is a popular delicacy in the Visayas. It is made from carabao's milk using salt and vinegar at levels that vary between manufacturers. Such variation results in differences in saltiness, acidity and sourness of the product.

Researchers Virchie Ann Chin and Dr. Roberta Lauzon of the Department of Food Science and Technology (DFST) in the Visayas State University (VSU) found out that the best formulation involved the use of 2 percent salt and 4 percent acid. Such formulation produced soft cheese which was most acceptable to the consumers.

The resulting soft white cheese costs Php 34.67 per 100 grams.

The researchers tested nine treatments, which were combinations of three levels of salt and three levels of acid. The most acceptable treatment formulation was determined through a sensory evaluation involving 48 panelists from the VSU community.

It used a 9-point Hedonic scale in evaluating sensory attributes including

color, saltiness, sourness, texture and overall acceptability. Contour plots were then generated from the data using predictive models to determine which treatment formulation was optimum.

The optimum formulation was further subjected to consumer preference test involving 100 consumers composed of college students and local residents of Baybay, Leyte.

The total percent moisture, ash, crude fat and crude protein contents were analyzed in the Animal Nutrition Laboratory of the Department of Animal Science of VSU following the standards of AOAC or Association of Official Analytical Chemists - International.

Analyses revealed that the end product contained 44.44% dry matter, 1.80% ash, 16.55% crude protein, 14.40% crude fat and 1.16% crude fiber.

The study was a collaborative undertaking of VSU and PCC at VSU.

Risk factors causing cryptosporidiosis...

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About 86% of the farm owners confessed that they do not practice proper waste disposal. Fifty-eight percent (58%) of these farms were found to have animals infected by the disease.

Most of these animals graze in open pasture which makes them prone to consume contaminated materials from improperly disposed waste. This waste may include human and animal excreta.

Some animals present in the area might also get infected due to the conditions in their area. The animals include cattle, goats, chicken and pigs. These animals can serve as hosts of infection to swamp buffaloes due to the presence of oocysts in their excreta, the study said.

Three percent of the farmers who own infected animals, usually dispose off their human waste by "balot" system or excreta wrapped in plastic and thrown to any area. The remaining 97% disposed their waste in open dumpsite or fields where their swamp buffaloes have access.

The study recommends that proper hygienic practices should be administered by the handler since humans can serve as hosts to transfer this disease. They could also be considered as one of the risk factors in increasing infection to other animals at risk.

Moreover, animals present in the farms other than swamp buffaloes can also be the sources of infection since cryptosporidiosis affect wider range of host.

The study likewise suggested that immediate action from the Provincial and Rural Health offices of Aurora Province should be employed due to the high prevalence of *Cryptosporidium* among the selected farms and given that diarrhea is second from the top ten leading reasons of morbidity in the province.

The study proposed that *Cryptosporidium* be included as one of the potential causes of diarrhea in Aurora Province.

OPINION



PCC as premier institution for livestock R&D

CLARO N. MINGALA, PhD
PCC National R&D Coordinator

The effort of PCC to capacitate its researchers through continuous training and advance studies are ways of harnessing the potential of its people to become more competent and productive scientists.

The establishment of the Philippine Carabao Center, as premier institution for livestock research and development (R&D) in the country, is indeed a proof of success in the rise from its humble beginning as a FAO-UNDP project in the 1980's to a highly recognized biotechnology center for livestock in the Philippines. The agency's efficient and productive execution of R&D activities truly follow the R&D cycle through effectiveness of studies, synthesis and theories, exploration and hypothesis, test and design as well as efficient implementation and improvement.

The effort of PCC to capacitate its researchers through continuous training and advance studies are ways of harnessing the potential of its people to become more competent and productive scientists. Various research disciplines comprised the R&D body of PCC, namely: the Biosafety and Animal Health, Nutrition, Molecular Genetics, Reproductive Biotechnology and the Socio-economics Research units. These units are the research workforce of the agency to look into the details that will contribute viable impacts to the livestock industry and help the rural farming communities.

Because of the worthwhile research outputs of PCC with its high caliber researchers and scientists, the Department of Agriculture entrusted recently to PCC an additional mandate spelled out under Administrative Order No. 9 issued by the Secretary of Agriculture, which is to

serve as the lead agency for ruminant biotechnology.

PCC strives to uplift its R&D efforts to meet global standards and become a world class research institute. The agency's R&D long-term plan encapsulates five major goals which are conserving indigenous genetic materials and improving the genetic potentials of the existing stocks, improving reproductive efficiencies, development of feeding and production systems for efficient and cost efficient production, safe environment, and improving income establishment of models for smallholder livestock entrepreneurs and continuously assess related policies.

The promotion of livestock development to provide opportunities for rural farming families toward improved nutrition and increased income has been the core goal of PCC and the agency's R&D direction guide. Expanding markets for livestock products, changing food quality and safety demands, new technologies and innovations all create both opportunities and challenges for reducing poverty through livestock development.

PCC and its partners, local and international collaborators, are continuously engaging themselves to beneficial researches in order to enable all stakeholders in livestock development to apply the tools of new science and technology in pursuit of a shared livestock R&D agenda.

R&D Highlights[©]

R&D Highlights, an annual publication of the Philippine Carabao Center, publishes in popularized form the agency's completed researches presented in its annual R&D Review. This publication reaches out to a wide scope of readers both in the science and non-science profession as well as the interested public.

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