Original Article

Molecular Characterization of Respiratory Bacterial Pathogens in Large and Small Ruminants

Gemerlyn Gonzales Garcia [1] Lawrence Pascual Belotindos [2]

Claro Niegos Mingala [2, 3*]

Abstract

Bacterial isolates from different cases of respiratory infections in small and large ruminants were evaluated using bacterial culture and semi-nested PCR and identification was confirmed by DNA sequencing. Universal primers targeting the bacterial 16S rRNA for bacteria were used. Other sets of primers that were specific for Gram-positive bacteria and combinations of primers specific for Gram-negative organisms were used in the second PCR. The amplified PCR products were subjected to DNA sequence analysis. The DNA sequences of the isolated bacteria were aligned with the DNA sequences of bacteria in the GenBank through BLAST. Results confirmed isolation of three Gram-positive and two Gram-negative organisms. Gene sequence studies demonstrated identification of the Gram-positive microorganisms as *Staphylococcus sciuri, Staphylococcus sporosarcinae* and *Bacillus pumilus* while the identified Gram-negative organisms were *Acinetobacter schindleri* and *Pseudomonas aeruginosa* from ruminants manifesting clinical signs of respiratory infection.

Keywords: *Acinetobacter schindleri, Bacillus pumilus,* DNA sequencing, *Pseudomonas aeruginosa, Staphylococcus sporosarcinae, Staphylococcu sciuri*

[1]*College of Veterinary Science and Medicine, Central Luzon State University, Science City of Muñoz 3120, Nueva Ecija, Philippines*

[2]*Philippine Carabao Center National Headquarters and Gene Pool, Science City of Muñoz 3120, Nueva Ecija, Philippines*

[3]*Affiliate Faculty, Department of Animal Science, Institute of Graduate Studies, Central Luzon State University, Science City of Muñoz 3120, Nueva Ecija, Philippines*

***Corresponding author: E-mail:** cnmingala@hotmail.com*

บทคัดย่อ

คุณลักษณะทางอณูชีววิทยาของเชื้อแบคทีเรียระบบทางเดินหายใจในสัตว์สี่กระเพาะขนาดเล็กและใหญ่

Gemerlyn Gonzales Garcia [1] Lawrence Pascual Belotindos [2] Claro Niegos Mingala [2, 3]*

ทำการแยกเชื้อแบคทีเรียจากสัตว์สี่กระเพาะขนาดเล็กและใหญ่ที่ติดเชื้อทางระบบทางเดินหายใจ โดยวิธีการเพาะแยกเชื้อ semi-nested PCR และการจำแนกเชื้อโดยการวิเคราะห์ลำดับเบสของเชื้อ ด้วยไพรเมอร์ต่อ 16S rRNAและใช้ชุดไพร์เมอร์ที่ความจำเพาะต่อแบคทีเรียแกรมบวกและแกรมลบ การวิเคราะห์ลำดับเบสของเชื้อแบคทีเรียผ่านธนาคารยีนด้วยโปรแกรม BLAST ผลการศึกษาพบแบคทีเรียแกรมบวก 3 สายพันธุ์ และแบคทีเรียแกรมลบ 2 สายพันธุ์ การวิเคราะห์ลำดับเบสสามารถจำแนกเชื้อแบคทีเรีย คือ แบคทีเรียแกรมบวกชนิด *Staphylococcus sciuri, Staphylococcus sporosarcinae* และ *Bacillus pumilus* แบคทีเรียแกรมลบชนิด *Acinetobacter schindleri* และ *Pseudomonas aeruginosa*

คำสำคัญ: *Acinetobacter schindleri Bacillus pumilus* การวิเคราะห์ลำดับเบส *Pseudomonas aeruginosa Staphylococcus sporosarcinae Staphylococcu sciuri*

[1] *College of Veterinary Science and Medicine, Central Luzon State University, Science City of Muñoz 3120, Nueva Ecija, Philippines*
[2] *Philippine Carabao Center National Headquarters and Gene Pool, Science City of Muñoz 3120, Nueva Ecija, Philippines*
[3] *Affiliate Faculty, Department of Animal Science, Institute of Graduate Studies, Central Luzon State University, Science City of Muñoz 3120, Nueva Ecija, Philippines*
***Corresponding author E-mail:** cnmingala@hotmail.com*

Introduction

Central Luzon, Philippines is an agricultural region where farmers raise cattle, water buffaloes, sheep and goats to augment family income. In raising these animals, a range of respiratory infections can be observed during inclement weather. Cases of respiratory infection in small and large ruminants are tentatively diagnosed based on the clinical signs observed in sick animals (VOR, 2012). Relying on the clinical signs without performing appropriate diagnostic procedures to identify the cause of the disease does not warrant full recovery in sick animals. Administering a medication without a definitive diagnosis may lead to unsuccessful treatment, further spread and aggravation of existing respiratory infection.

Respiratory infections among ruminants raised in local field conditions is not well documented in spite of a pool of overseas report that describe the susceptibility of cows and goats to Staphylococcus infections (Kleine et al., 2010; and Erickson et al., 2013), sheep receptivity to infections caused by *Bacillus pumilus* (Tena et al., 2007), vulnerability of animals to Acinetobacter infections (Garnacho et al., 2003; Allen and Hartman, 2005), conditions marked by inflammation and sepsis associated with *Pseudomonas aeruginosa* (Leitner and Krifucks, 2007) and other respiratory disease syndromes in cattle (Dariusz et al., 2012) and in young dairy calves (Garcia and Daly, 2010).

A method that validates the existence of respiratory infections in ruminants is necessary as a reference in initiating measures that prevent the spread of infection through the oral route when ruminants graze on pastures. As there is a limited source of information on the involvement of bacteria in respiratory infections of ruminants raised in different farming conditions in the region, conventional analysis of samples from the respiratory system in adjunct to a DNA-based protocol was applied to identify bacteria and confirm diagnosis of respiratory infection. A research work was undertaken to look into the relevance of PCR as a recognition system for bacteria that are associated with respiratory ailments in ruminant. The delivery of a fast, sensitive and accurate result of disease detection is a practical approach in planning for treatment and in strengthening the diagnostic component of existing animal health programs.

Materials and Methods

Criteria in the selection of animals used in the study: Ruminants (cattle, buffaloes, goats and sheep, 5-head each) from different farms showing signs of respiratory distress for two to three days and did not receive medication as reported by owners and caretakers during the interview were considered as sources of sample for examination. Animals with respiratory infection accompanied by a fever as indicated by a body temperature above 40^0C, excessive viscous mucus secretions from the nostrils, bloody diarrhea and cyanotic mucus membranes upon physical examination were selected for the study.

Collection of samples for bacteriological examination : Four cotton tipped swabs were used in taking samples from both nostrils of animals with the above clinical signs. The swabbed samples were temporarily held in a test tube containing normal saline, placed in test tube racks and held in Styrofoam boxes during transport to the laboratory.

Bacterial cultivation and DNA extraction: The protocols of Gabinaitiene et al. (2011) and Kleine et al. (2010) were adapted in the cultivation of bacteria with slight modifications. Briefly, the samples in normal saline were inoculated in blood agar plates then incubated at 37^0C for 24 hours. Colonies of bacteria that showed different colony characteristics and growth patterns in blood agar after incubation were picked up separately with the use of a sterile inoculating loop and representative smears of each colony were made to evaluate Gram stain reactions. Different bacterial colonies were separately isolated and transferred in Trypticase Soy Agar (TSA), incubated at 37^0C for 24 hours before evaluation of biochemical characteristics (GEN III Microbial Identification System, Biolog).

Pure culture of each bacterium was transferred in 1 ml Luria-Bertani (LB) broth containing 50% glycerol in 1.5 ml tubes before DNA extraction. The samples were incubated with shaking (200 rpm) at 37^0C for 16 hours. After incubation, the samples were centrifuged at 14000 rpm for 1 min. The supernatants were decanted while the pellets were vortexed. Cell lysing solution (700 µl per tube) was added to the samples and vortexed. The samples were again centrifuged at 14000 rpm for 1 min and the supernatants were discarded. The pellets were vortexed before adding cell lysing solution (600 µl per tube). The samples were again centrifuged at 14,000 rpm for 1 min before adding the nuclei lysing solution. The samples were vortexed before adding the protein precipitating solution (100 µl per tube). The samples were again vortexed and centrifuged at 14,000 rpm for 10 min and after which the supernatants were collected. Isopropanol (500 µl) was added to each sample and gently mixed before centrifugation at 14,000 rpm for 1 min. The supernatants were discarded before the addition of 500 µl 70% ethanol. The samples were again centrifuged at 14000 rpm for 1 min and the pellet was allowed to dry for 1 hour. The dried DNA samples were rehydrated with 50 µl DNA rehydration solution and used for PCR.

Primers for PCR: A pair of universal primers (NF and NR) targeting the 16S rRNA gene of most bacteria was used in PCR adapting the protocol of Carroll et al. (2000). The forward primer (NF, 5′ GGCGGCAK GCCTAAYACATGCAAGT 3′)) and the reverse primer (NR, 5′ GACGACAGCCATGCAS CACCTGT 3′) are expected to amplify DNA of many bacteria. Another pair of primers which included the forward primer (P2F) 5′ GCGRCTCTCTGGTCTGTA 3′ and a copy of the reverse primer (NR) from the universal primer pair was applied in the next PCR which is expected to amplify DNA of Gram-positive bacteria. A third set of primers included a copy of the forward primer (NF) 5′ GGCGGCAKGCCTAA YACATGCAAGT 3′ from the universal primer paired with (N6R) 5′ GTTCCCGAAGGCACC 3′. This set of primers is expected to amplify DNA of Gram-negative bacteria. The reaction mixture contained 11.3 µl sterile DDW, 4 µl 5 times PCR buffer, 2 µl MgCl2, 0.5 µl DNTP, 0.5 µl forward primer, 0.5 µl reverse primer, 0.2 µl TAQ, and 2 µl DNA template making up a total volume of 20 µl.

DNA amplification through PCR: PCR was carried out using a thermocycler which was programmed to function with an initial denaturation at 94^0C for 5 min, denaturation at 94^0C for 30 sec, annealing at 50^0C for 30 sec and extension at 72^0C for 30 sec, performed in 35 cycles. Final extension was done at 72^0C for 2 min and final hold at 4^0C.

Gel electrophoresis of PCR products: PCR products were resolved in 2% agarose gel in 25 ml 1x TAE buffer. PCR products (3.0 µl) and the bp marker (5 µl) were loaded to the wells. Electrophoresis was undertaken for 30 min in a 10-volt electric current. PCR products were visualized and photographed in a UV trans-illuminator (Gel Doc, USA).

DNA sequencing: PCR products were purified and sequenced (AsiaGel, Malaysia) before alignment. The identity of each isolate was confirmed through BLAST made after comparison of aligned sequences with those of bacteria in the GenBank which had similar DNA homologies.

Results

Results demonstrated that from the 80 samples collected, there was a total of 42 bacterial isolates coming from cattle (10), buffaloes (14), sheep (8) and goats (10) (Table 1). Based on Gram stain reactions and biochemical profiles of bacterial isolates, three species of Gram-positive bacteria were recognized as *Staphylococcus sciuri*, *Staphylococcus sp*. and *Bacillus pumilus* while two were Gram-negative recognized as *Acinetobacter schindleri* and *Pseudomonas sp*.

Result of PCR show that in using the universal primers NF/NR, DNA products were detected with an amplicon size of 1025 bp (Fig 1). This finding indicated that the DNA products from all samples evaluated were of bacterial origin. In a second PCR run where specific primers for Gram-positive bacteria (P2F/NR) were applied, DNA products with amplicon size of 355 bp were detected (Fig 2). Results validated a prior observation on 3 possible bacterial isolates from ruminant samples with Gram-positive reaction.

Table 1 Samples for microbiological examination

Animal source	Number of samples collected	Number of isolates	Identified bacterial isolates
Cattle	20	10	*A. schindleri [6],* *S. sporosarcinae[2],* *B. pumilus [2]*
Buffaloes	20	14	*S. sporosarcinae [5],* *P. aeruginosa [5],* *B. pumilus [2],* *S. sciuri [2]*
Sheep	20	8	*B. pumilus [5],* *P. aeruginosa [1],* *S. sciuri [2]*
Goats	20	10	*S. sciuri [7],* *B. pumilus [2],* *P. aeruginosa [1]*
Total	**80**	**42**	

S. sporosarcinae (*Staphylococcus sporosarcinae*),
S. sciuri (*Staphylococcus sciuri*), *B. pumilus* (*Bacillus pumilus*),
A. schindleri (*Acinetobacter schindleri*),
P. aeruginosa (*Pseudomonas aeruginosa*).

In utilizing another set of primers (NF/N6R) intended to recognize DNA from Gram-negative bacteria in the next PCR, DNA products with amplicon size of 985 bp were demonstrated from two bacterial isolates (Fig 3). This data provided a confirmation of a previous observation on two possible Gram-negative bacteria as isolates from ruminant samples.

A summary of the DNA sequences of the bacterial isolates derived after alignment and identification through BLAST is shown in Table 2. These data revealed that the three Gram-positive organisms exhibiting DNA products with a molecular weight of 355 bp during PCR that applied the P2F/NR primer combination were identified as *Staphylococcus scuiri, Staphylococcus sporosarcina* and *Bacillus pumilus.* In addition, the two Gram-negative organisms demonstrating DNA products with a molecular size of 985 bp during PCR that applied the NF/ N6R primer combination were identified as *Acinetobacter schindleri and Pseudomonas aeruginosa.*

Based on these data, the nasal cavity of cattle in course of respiratory infection was predominated by *A. schindleri* (6 isolates), *S. sporosarcina* (2 isolates) and *B. pumilus* (2 isolates) while *P. aeruginosa* and *S. sporosarcina* (5 isolates each) outweighed *B. pumilus* (2 isolates) and *S. scuiri* (2 isolates) in water buffaloes. The nasal cavity of goats in the track of respiratory infection was inhabited by *S. scuiri* (7 isolates), *B. pumilus* (2 isolates) and *P. aeruginosa* (1 isolate) while those of sheep were primarily colonized by *B. pumilus* (5 isolates), *P. aeruginosa* (1 isolate) and *S. scuiri* (2 isolates) (Table 1).

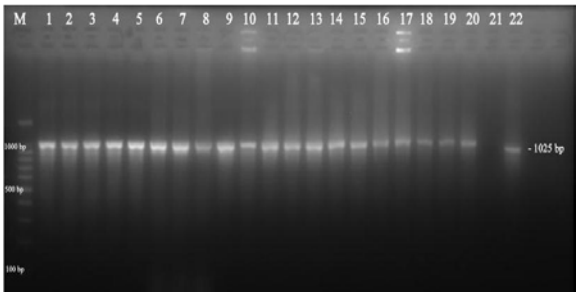

Figure 1 Gel electrophoresis of PCR products from bacterial isolates with the use of the universal primers (NF/NR). Lanes 1 to 3 (*Staphylococcus sciuri*), Lanes 4 to 6 (*Staphylococcus sporosarcina*), Lanes 7 to 9 (*Bacillus pumilus*), Lanes 10 to 12 (*Staphylococcus sciuri*), Lanes 13 to 16 (*Acinetobacter schindleri*), Lanes 17 to 20 (*Pseudomonas aeruginosa*), Lane 21 (Negative control, DDW), Lane 22 (Positive control, *E. coli*). Lane M (100 bp DNA marker, 100 ng/µl).

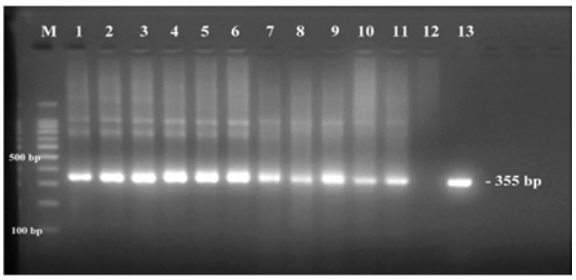

Figure 2 Gel electrophoresis of PCR products from bacterial isolates with the use of the Gram-positive bacterial primers (P2F/NR). Lanes 1 to 3 (*Staphylococcus sciuri*), Lanes 4 to 6 (*Staphylococcus sporosarcinae*), Lanes 7 to 9 (*Bacillus pumilus*), Lanes 10 to 11 (*Staphylococcus sciuri*), Lane 11 (Negative control, DDW), Lane 12 (Positive control, *Lactobacillus* sp.), Lane M (100 bp DNA marker, 100 ng/µl).

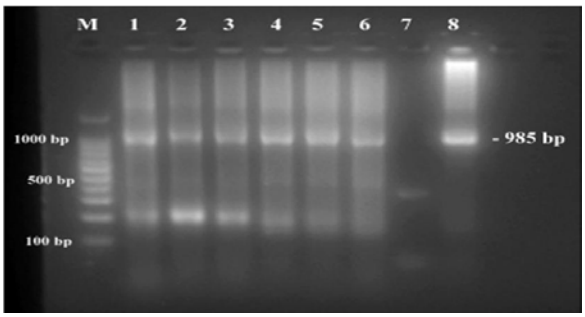

Figure 3 Gel electrophoresis of PCR products from bacterial isolates with the use of a Gram-negative bacterial primers (NF/NGR). Lanes 1 to 3 (*Acinetobacter schindleri*), Lanes 4 to 6 (*Pseudomnas aeruginosa*), Lane 7 (Negative control, DDW), Lane 8 (Positive control, *E. coli*), Lane M (100 bp DNA marker, 100 ng/µl).

Table 2 DNA sequences of bacterial isolates from ruminants with respiratory infections (AsiaGel, Malaysia)

Bacterial isolate	DNA Sequences	Amplicon size (bp)
Staphylococcus spp.	**GTGACTCTNTGGTCTGTA**ACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCT GGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGATAAC GCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGC ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTG AAAACTCTAGAGATAGAGCTTTCCCCTTCGGGGG**ACAAAGTGACAGGTGGTGCATGGTGTGTC**	337
S. sciuri, Native goat	**GCGGTAGTGAACGTGGGGATCACAGGATTAGATA**CCCTGGTAGTCCCGCCGTAAACGATGAGTGCTA AGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGAC CGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA AGCAACGCGAAGAACCTTACCAAGTCTTGACATCCTTTGACCGCTCTAGAGATAGAGTCTTCCCCTTCGG GGG**ACAAAGTGACGGGTGGTGCGTGGCTGTCC**	306
S. sciuri, Boer goat	**ACAGGATTAGATACCCTGGTAGTC**CACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCC CTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGG AATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC AAATCTTGACATCCTTTGAAAACTCTAGAGATAGAGCTTTCCCCTTCGGGGGACAAAGTG**ACAGGTGGT GCATGGCTGTCGTC**	287
B. pumilus	**GCGACTCTCTGGTCTGTA**ACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC TGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAAC GCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGC ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTG ACAACCCTAGAGATAGGGCTTTCCCTTCGGGG**ACAGAGTGACAGGTGGTGCATGGCTGTCGTC**	337
A. schindleri	**GGCGAACAGGTGAGTAACNTCNCGAAANTNCGTGATAGANGGGGATAACTACNGGAAANGGTNG NTAATATTTTCATANCGTCGCNTTACCANTGAGAGGGNNCT**GGCCTAACACATGCAAGTCGAGCGGG GAAGGTTGCTTCGGTAACTGACCTAGCGGCGGACGGGTGAGTAATGCTTAGGAATCTGCCTATTAGTGG GGGACAACGTTCCGAAAGGAACGCTAATACCGCATACGCCCTACGGGGGAAAGCAGGGGATCTTCGGA CCTTGCGCTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGA TCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTTGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTC CTTTAGTTAATACCTAAAGAGAGTGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGTGCCAGCAG CCGCGTACTGGAAAGCTAGAGTATGGGAGAGGATGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAG AGATCTGGAGGAATACCGATGGCGAAGGCAGCCATCTGGCCTAATACTGACGCTGAGGTACGAAAGCA TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGTCTACTAGCCGTTGGGGCC TTTGAGGCTTTAGTGGCGCAGCTAACGCGATAAGTAGACCGCCTGGGGAGTACGGTCGCAAGACTAAA ACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAG AACCTTACCTGGCCTTGA**GGTGAAATGACGTAAAGATATGGAGGAACCCAGTGGCGAAGGCGACTT TCTGGTCTGTAAC**	864
P. aeruginosa	**GGGAATTTAAGCATGTCAGGCCAGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCA CCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGT**TTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGA CTTATCGCGTTAGCTGCGCCACTAAGATCTCAAGGATCCCAACGGCTAGTCGACATCGTTTACGGCGTGG ACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATCAGTCCAGGTGGT CGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATTCCACCACCCTCT ACCGTACTCTAGCTTACCAGTTTTGGATGCACTTCCCAGGTTGAGCCCGGGGATTTCACATCCAACTTGC TGAACCACCTACGCGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCCCCCTTCGTATTACCGCGGCT GCTGGCACGAAGTTAGCCGGTGCTTATTCGGTTGGTAACGTCAAAGCAGCAAGGTATTAACTTACTGCTC TTCCTCCCAACTCAAAGTGCTTTACAATCCGAAAACCTTCTTCACACACGCGGCATTGCTGCATCAGGCT TTCGTCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTG ACTGATCATCCTCTCAGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTA ACTGTTAGGATGNTTCCGCCCCTAATGCTGCACTAACCATTAGGCGATCCGCCTGGGGGAGTACGGNCN CAAGATTAAAACTCAANNAATTGACGGGGCCCGCACAAGC**GGTGNNCATGTAGNTTNATTCAAAGC ANCNCNAGAACCTTACCNGGCCNTGACATGC**	932

Discussion

The study identified 5 species of bacteria that went along with respiratory infections of ruminants. Identification of bacteria with the use of conventional and DNA-based protocols assisted in coming up with a confirmatory diagnosis of respiratory infections. Amplification of DNA from bacterial isolates by PCR that made use of universal primers NF and NR and specific primers P2F and N6R paired with either of the two universal pairs of primers were found to be efficient in the detection of bacterial isolates. PCR has been in extensive use in the detection of infectious diseases (Clarridge, 2004), bacterial DNAs in pleural fluids (Cremades et al., 2011), Pseudomonas infections (Lodeng et al., 2006; and Lavenir et al., 2007) and in the differentiation of Gram-positive and Gram-negative bacteria utilizing the sets of primers (Carrol et al., 2000) employed in this study. DNA sequencing that confirms identity of specific bacteria in bovine respiratory tract (Gabinaitiene et al., 2011), Staphylococci in food-borne (Landeta et al., 2011; and Irlinger et al., 2012) and clinical infections (Couto et al., 2000; Novakova et al., 2006; Kleine et al., 2010), molecular identification of Acinetobacter species (La Scola et al., 2006), identification of Bacillus species in skin infections (Tena et al., 2007) and other bacterial pathogens (Kolbert and Persing, 1999) have been reportedly undertaken.

The isolation and identification of bacteria during the course of respiratory infection in ruminants described in this paper is a preliminary attempt to document this information in the absence of a previous record. These data explain the possible participation of these bacteria in the process of respiratory infection in ruminants. Several accounts on the pathogenicity of bacteria like the ones isolated have been cited. The involvement of Staphylococcus has been described in urinary and other clinical infections in animals and humans (Novakova et al., 2006; Chen et al., 2007; Ferreira et al., 2012; Erickson et al., 2013). The pathogenic participation of *B. pumilus* in cutaneous infections has been discussed (Tena et al., 2007). The opportunistic nature of *Acinetobacter sp.* explains the participation of the bacterium in

pneumonia cases (Garnacho et al., 2003), in bacteremia (Loubinoux et al., 2003), and in respiratory and eye infections (Allen and Hartman, 2005). Conditions marked by general inflammation, sepsis, gangrenous infections and mastitis have been claimed to be mediated by *Pseudomonas aeruginosa* (Leitner and Krifucks, 2007).

The results of the study provide evidence that PCR facilitates that diagnosis of respiratory bacterial infections in ruminants and DNA sequencing proves to be the ultimate arbiter in bacterial identification (Kolberg and Persing, 1999; Clarridge, 2004; Cremades et al., 2011; Al-Khaldi et al., 2012). This study presents initial information on DNA sequences of bacterial isolates from ruminants with respiratory infections.

Conclusion

Based on the above findings, potential microbial pathogens that accompany respiratory infections in ruminants can be isolated using basic microbiological *in vitro* protocols and the identity of which can be evaluated by PCR and confirmed by DNA sequencing. It is seen that the integration of PCR-based methods in laboratory diagnostic systems harnesses the competence of existing animal health programs.

Acknowledgements

We thank the following people: Drs. Hershe A. Bautista, Ronald Buenne A. Reyes, Gerald L. Ordonez, Gia Victoria Mari B. Marcelo, Rose Anne C. del Pilar, Josiah Gene A. Tolentino, for their technical assistance and help in preparing the manuscript. Appreciation is also extended to Dr. Libertado C. Cruz, Executive Director of the Philippine Carabao Center, for his valuable support.

References

Al-Khaldi SF, Mossoba MM, Allard MM, Lienau EK and Brown ED 2012. Bacterial identification and subtyping using DNA microarray and DNA sequencing. Methods Mol Biol. 881: 73- 95.

Allen DM and Hartman BJ 2005. *Acinetobacter* species, In: Principles and practice of infectious diseases. Vol 2, 6th ed. GL Mandell, JE Bennett and R Dolin (eds). Elsevier Churchill Livingstone, Philadelphia : 2632-2636.

Carroll NM, Jaeger EEM, Choudhury S, Dunlop AAS, Matheson MM, Adamson P and Okhravi N 2000. Detection and discrimination between Gram-positive and Gram-negative bacteria in intraocular samples using nested PCR. J Clin Microbiol. 38: 1753-1757.

Chen S, Wang Y, Chen F, Yang H, Gan M and Zheng SJ 2007. A highly pathogenic strain of *Staphylococcus sciuri* caused fatal exudative epidermitis in piglets. PLoS One. 2: e147.

Clarridge JE 2004. Impact of 16S rRNA gene sequence analysis or identification of bacteria on clinical microbiology and infectious diseases. Clin Microbiol Rev. 17: 840-862.

Couto I, Sanches IS, Sa-Leao R and De Lencastre H 2000. Molecular characterization of *Staphylococcus sciuri* strains isolated from humans. J Clin Microbiol. 38: 1136-1143.

Cremades R, Galiana A, Rodriguez JC, Santos A, Lopez P, Ruiz M, Garcia-Pachon E and Royo G 2011. Identification of bacterial DNA in noninfectious pleural fluid with a highly sensitive PCR method. Respiration. 82: 130-135.

Dariusz B, Szymanska-Czerwinska M and Dudek K 2012. "Bovine Respiratory Syndrome (BRD) Etiopathogenesis, Diagnosis and Control". In: A Bird's-Eye View of Veterinary Medicine. C.C. Perez-Marin (ed). [Online]. Available: http://cdn.intechopen.com/pdfs/28682/InTech Bovine_respiratory_syndromebrd_etiopathogenesis_diagnosis_and_control.pdf. Accessed March 28, 2013.

Eriksson A, Giske CG and Ternhag A 2013. The relative importance of *Staphylococcus saprophyticus* as a urinary tract pathogen: distribution of bacteria among urinary samples analysed during 1 year at a major Swedish laboratory. APMIS. 121: 72-78.

Ferreira AM, Bonesso MF, Mondelli AL, da Cunha Mde L 2012. Identification of *Staphylococcus saprophyticus* isolated from patients with urinary tract infection using a simple set of biochemical tests correlating with 16S-23S interspace region molecular weight patterns. J Microbiol Methods. 91: 406-411.

Gabinaitiene A, Siugzdaite J, Zilinskas H, Siugzda R and Petkevicius S 2011. Mycoplasma bovis and bacterial pathogens in the bovine respiratory tract. Veterinarni Medicina. 56: 28–34.

Garcia A and Daly R 2010. "Respiratory Disease in Young Dairy Calves". [Online]. Available: http://www.sdstate.edu/vs/extension/dairy/upload/ExEx4045_Resp-Dis-Dairy-Calves.pdf. Accessed May 11, 2012.

Garnacho J, Sole-Violan J, Sa-Borges M, Diaz E and Rello J 2003. Clinical impact of pneumonia caused by *Acinetobacter baumannii* in intubated patients: a matched cohort study. Crit Care Med. 31: 2478-2482.

Irlinger F, Loux V, Bento P, Gibrat JF, Straub C, Bonnarme P, Landaud S and Monnet C 2012. Genome sequence of *Staphylococcus equorum subsp. equorum* Mu2, isolated from a French smear-ripened cheese. J Bacteriol. 194: 5141-5142.

Kleine B, Gatermann S and Sakinc T 2010. Genotypic and phenotypic variation among *Staphylococcus saprophyticus* from human and animal isolates. BMC Res Notes. 3: 163.

Kolbert CP and Persing DH 1999. Ribosomal DNA sequencing as a tool for identification of bacterial pathogens. Curr Opin Microbiol. 2: 299-305.

La Scola B, Gundi VA, Khamis A and Raoult D 2006. Sequencing of the *rpoB* gene and flanking spacers for molecular identification of *Acinetobacter* species. J Clin Microbiol. 44: 827-832.

Landeta G, Reverón I, Carrascosa AV, de las Rivas B, Muñoz R 2011. Use of recA gene sequence analysis for the identification of *Staphylococcus*

equorum strains predominant on dry-cured hams. Food Microbiol. 28: 1205-1210.

Lavenir R, Jocktane D, Laurent F, Nazaret S and Cournoyer B 2007. Improvement reliability of *Pseudomonas aeruginosa* PCR detection by the use if species-specific ecfX gene target. J Microbiol Methods. 70: 20-29.

Leitner G and Krifucks O 2007. *Pseudomonas aeruginosa* mastitis outbreaks in sheep and goat flocks: Antibody production and vaccination in a mouse model. Vet Immunol Immunopathol. 119: 198-203.

Leroy S, Lebert I, Chacornac JP, Chavant P, Bernardi T and Talon R 2009. Genetic diversity and biofilm formation of *Staphylococcus equorum* isolated from naturally fermented sausages and their manufacturing environment. Int J Food Microbiol. 134: 46-51.

Lodeng AG, Ahlen C, Lysvand H and Iversen OJ 2006. Development and evaluation of a new PCR assay for detection of *Pseudomonas aeruginosa* D genotype. Clin Microbiol Infect. 12: 761-768.

Loubinoux J, Mihaila-Amrouche L, Le Fleche A, Pigne E, Huchon G, Grimont PA and Bouvet A 2003. Bacteremia caused by *Acinetobacter ursingii*. J Clin Microbiol. 41: 1337-1338.

Nováková D, Sedlácek I, Pantůcek R, Stetina V, Svec P and Petrás P 2006. *Staphylococcus equorum* and *Staphylococcus succinus* isolated from human clinical specimens. J Med Microbiol. 55: 523-528.

Tena D, Martinez-Torres JA, Perez-Pomata MT, Sáez-Nieto JA, Rubio V and Bisquert J 2007. Cutaneous infections due to *Bacillus pumilus*. Clin Infect Dis. 44: e40-e42.

VOR 2012. Veterinary Office Report. Office of the Provincial Veterinarian. Province of Nueva Ecija. Philippines.